PHYSIOLOGY OF THE INCOMPATIBILITY FACTORS IN SCHIZOPHYLLUM COMMUNE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 618

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Introduction

A pattern of sexuality involving multiple incompatibility alleles at two loci was first described by KNIEP (16) in the hymenomycete *Schizophyllum commune* in 1920. He showed that mating type in *S. commune* is controlled genetically by two series of incompatibility alleles at distinct loci and that every fruit is heterozygous at both loci, therefore giving rise to spores of four different mating types. The heterozygous condition of the fruit follows necessarily from the fact that two mycelia are compatible and produce fruit only when their mating types are such that they differ in the alleles at both loci.

Elucidation of this complex sexual system by KNIEP was followed during the next 15 years by a large number of publications in various countries on the manifestations of sex in the basidiomycetes. His findings were confirmed and extended to many other organisms. About 90% of the hymenomycetes have been found to be heterothallic and, of these, 61% are tetrapolar, their fruits giving rise to spores of four different mating types, as do those of *S. commune;* the other 39% are bipolar—that is, they have only one series of incompatibility alleles, and their fruits give rise to spores of only two different mating types (31).

The extensive work done subsequently to KNIEP's publications was, however, disappointing in many respects, for, with the exception of the "Buller phenomenon," no new fundamental knowledge was added to his account.

Recently the genetics of microörganisms has aroused considerable interest, and many old data have been re-examined. WHITEHOUSE (31) has summarized many published accounts to date on the multiple incompatibility alleles among the fungi. A system of multiple alleles controlling compatibility is widespread among the hymenomycetes, the gasteromycetes, and the angiosperms. In all these diverse groups the essential pattern is the same: where two identical alleles are brought together, there is one kind of reaction; where two different alleles are brought together, there is a different reaction. Furthermore, it appears to be irrelevant which two of the many possible combinants are actually involved. In the hymenomycetes the number of different alleles at each locus present in natural populations has been estimated roughly at one hundred (31); in the gasteromycetes it would appear that the number is much smaller.

A variation of the multiple-allele system among the hymenomycetes has been reported for *Solenia anomala* (11). Here, apparently, a multiple-allele system coexists with a two-allele system. The alleles of the two-allele system control the size of the mycelium as well as compatibility, and mycelia can, therefore, be divided into two types, large and small. Mating occurs only between a large and a

small mycelium, the smaller plant always diploidizing the larger.

The only theory which has been put forward to account for the peculiarities of the multiple-allele system, as found in the higher fungi, is that of HARTMANN (13), who incorporated the phenomena found among the basidiomycetes into his general theory of sexuality. His best evidence appears in this behavior of *Solenia*, but his theory is still not of very great value in elucidating either the genetics or the physiology of sex in the basidiomycetes.

DARLINGTON and MATHER (7) and others have suggested that the multiple-allele system, both in the angiosperms and in the fungi, depends on some type of antigenic reaction. Unfortunately, the evidence for such a reaction is very tenuous as regards the angiosperms (19) and is, at present, nonexistent as regards the fungi. In view of the lack of any other hypothesis, however, one involving such a reaction, in which the difference between the various alleles would depend on a complementariness of some large protein or polysaccharide molecule rather than on any chemical property involving a valency bond, remains attractive.

Whatever the nature of the processes involved may be shown to be, a factor with such a large number of alternate states controlling a sexual process presents many interesting problems. These problems fall into two main categories: (*a*) the manner in which these genes inhibit or promote the series of events leading to fruiting and the formation of basidiospores and (*b*) the origin of so large a number of different alleles and their maintenance in a population. Data bearing on the first of these will be described here. They consist of two main interrelated studies: (*a*) occurrence and properties of heterokaryons among various incompatible pairs of mycelia (22) and (*b*) physiology of a mutant which exhibits a peculiar mating behavior.

Nomenclature, material, and methods

In the present work the techniques and materials which have been employed correspond to those used by previous workers. Some new techniques and modifications of old ones, however, require description.

The basic medium used in all experiments, unless otherwise stated, had the following composition:

Glucose	2.0 %
Peptone	0.2 %
$K_2H_4PO_4$	0.2 %
$MgSO_4$	0.02%
$FeCl_3$	One drop of a 2% solution per liter
Agar	2.0 % when required

The pH value of this medium was 5.3 before the addition of agar and was 6.3 with the agar.

All matings were made on solid medium in Petri dishes. Inocula consisted of small agar cubes about 2 mm. on a side, cut from the periphery of a culture. Inoculations for each mating were made about 5 mm. apart unless otherwise stated.

Fruiting-bodies, whether wild or grown in the laboratory, are numbered consecutively in Roman numerals. In the case of each wild fruiting-body an abbreviation of the locality from which it was collected follows the Roman numeral. Cultures derived from a given fruiting-body, often unfortunately referred to as a "geographical race," are here designated as belonging to a "stock."

During the first part of the work to be described only one stock was cultured in the laboratory. This stock was derived from I (arg), collected in 1947 in the

Argonne Forest Preserve, Cook County, Illinois, by Dr. J. R. RAPER. Later two more stocks were brought into culture: XV (ad), collected in 1949 in the Adirondack Mountains, New York, by Dr. RAPER, and XXV (mad), collected in 1949 near Madison, Wisconsin, by the author. No spores from any other wild fruiting-bodies were handled in the laboratory during the course of the work.

The alleles for incompatibility found in these stocks are designated by capital letters with different superscripts. Thus, from fruiting-body I (arg) the alleles A^1, A^2, B^1, and B^2 were obtained; from XV (ad) the alleles A^3, A^4, B^3, and B^4; and from XXV (mad) the alleles A^5, A^6, B^1, and B^6. The first and third of these stocks have an allele (B^1) in common.

It is furthermore proposed that (*a*) any mycelium containing only one kind of nucleus, which has hitherto been variously designated a primary mycelium, a haploid mycelium, and a single-spore mycelium, be known as a "monokaryotic mycelium," or a "monokaryon"; (*b*) any mycelium containing two or more different nuclei be known as a "heterokaryotic mycelium," or a "heterokaryon"; and (*c*) any mycelium having a pair of compatible nuclei, hitherto referred to as a secondary mycelium, a diploid mycelium, or a dikaryon, be known by the latter term, i.e., a "dikaryotic mycelium," or a "dikaryon." For convenience, the terms "dikaryotic mycelium" and "dikaryon" are used synonymously throughout the paper.

Matings are represented in the customary way by a "×" sign between the symbols representing the two, or more, mycelia mated. If an inoculum was taken from one mycelium of a mating and was mated in turn with another mycelium, the first mating is inclosed in parentheses and the particular mycelium from which the inoculum was taken is shown by an underline. Thus, $(A^1B^2 \times \underline{A^1B^1}) \times A^2B^1$ means that a mating between monokaryons A^1B^2 and A^1B^1 was made and that a subculture from the A^1B^1 side of this mating was subsequently mated with A^2B^1. Any additional matings are shown by the use of brackets and a double underline. Thus, $[(A^1B^2 \times \underline{\underline{A^1B^1}}) \times A^2B^1] \times A^1B^2$ would mean that from the $(A^1B^2 \times A^1B^1) \times A^2B^1$ mating explained above a subculture was taken from the $A^1B^2 \times A^1B^1$ side and mated with an A^1B^2 monokaryon.

The constitution of the mycelia resulting from compatible matings, i.e., dikaryons, is symbolized by a "+" sign instead of an "×" between the symbols representing the monokaryons concerned. A "+" sign is not used, however, in the case of other types of heterokaryons.

Some new terms have been devised to designate matings showing the behavior discovered by the late Dr. BULLER and commonly referred to as the "Buller phenomenon." This concerns the diploidization of a monokaryon by a dikaryon. Such matings will be referred to in this report as "di-mon" matings. There are three categories of di-mon matings which need to be distinguished: (*a*) Matings of the type $(A^1B^1 + A^2B^2) \times A^1B^1$, in which one of the two nuclei of the dikaryon is compatible with the nucleus of the monokaryon—called "legitimate" by Dr. BULLER—will be termed "hemi-compatible" di-mon matings. (*b*) Matings of the type $(A^1B^1 + A^2B^2) \times A^1B^2$—called "illegitimate" by Dr. BULLER because neither of the nuclei of the dikaryon is compatible with that of the monokaryon—will be called "noncompatible" di-mon matings. (*c*) Matings of the type $(A^1B^1 + A^2B^2) \times A^3B^3$, in which both nuclei of the di-

karyon are compatible with the nucleus of the monokaryon, will be known as "compatible" di-mon matings.

It is realized that many objections can be raised to the introduction of these terms (5) and also that should new techniques be used in subsequent work the terms may have to be altered or amplified. They have been found convenient, however, in describing the experiments performed, make the maximum use of terms already in common usage without resorting to slang, and are reasonably euphonious.

All cytological observations described in this report were made with the use of a phase-contrast microscope.

A report on genetic experiments which were made concurrently with the present work will be published elsewhere together with a description of the crosses which were made in order to obtain the monokaryons of various mating types used in the experiments reported here.

Results

The experimental results comprise several distinct categories: (*a*) different types of interactions of monokaryons having similar or dissimilar incompatibility alleles; (*b*) patterns of nuclear behavior in di-mon matings; (*c*) origin, morphology, and physiology of an anomalous type of monokaryon; and (*d*) pattern of diploidization in compatible di-mon matings.

Types of reaction

The four reactions which occur when a monokaryon is mated with each of the four incompatibility types of the same stock have been found to be distinguishable (fig. 1). When the alleles at both of the incompatibility loci are different, a typical dikaryotic mycelium is formed. When the alleles at both loci are the same, there is a region of overlap where the two mycelia meet. When the alleles at the *B* locus are the same and those at the *A* locus are different, a "barrage" is formed which appears as a line of poor growth at the junction of the two mycelia. When the alleles at the *A* locus are the same and those at the *B* locus are different, there is developed a characteristic growth throughout the two mycelia of the mating. This growth may be of normal extent, but it is thin and has very poorly developed aerial hyphae; it has been named "flat."

The types of interaction between the monokaryons of the four mating types derived from a single fruit are shown in table 1. It is clear that, when these different patterns are recognized, the naming of factors from a new fruit is no longer arbitrary but that each can be definitely designated as *A* or *B*.

These four basic patterns of response between monokaryons are described in detail below, but first the results of a few preliminary experiments are given. These experiments were designed to find out whether any filterable substances were responsible for the different mating patterns. They were performed on solid and on liquid media. In liquid media the four monokaryons A^1B^1, A^2B^2, A^1B^2, and A^2B^1 were grown in filtrates of media in which the monokaryons A^1B^1 and A^1B^2 had grown. On solid media various monokaryons were grown on both sides of cellophane membranes, or monokaryons were grown on agar over which another monokaryon had grown on a cellophane membrane and had been removed with the membrane. No significant differences in growth rate or in general appearance were observed in any of these experiments. Microscopically the hyphae resembled those of normal monokaryons

except in the case of two monokaryons of type A^1B^1 grown in filtrates of A^1B^2, the hyphae of which had numerous knobs and short branches. Such hyphae, as will be seen below, are characteristic of

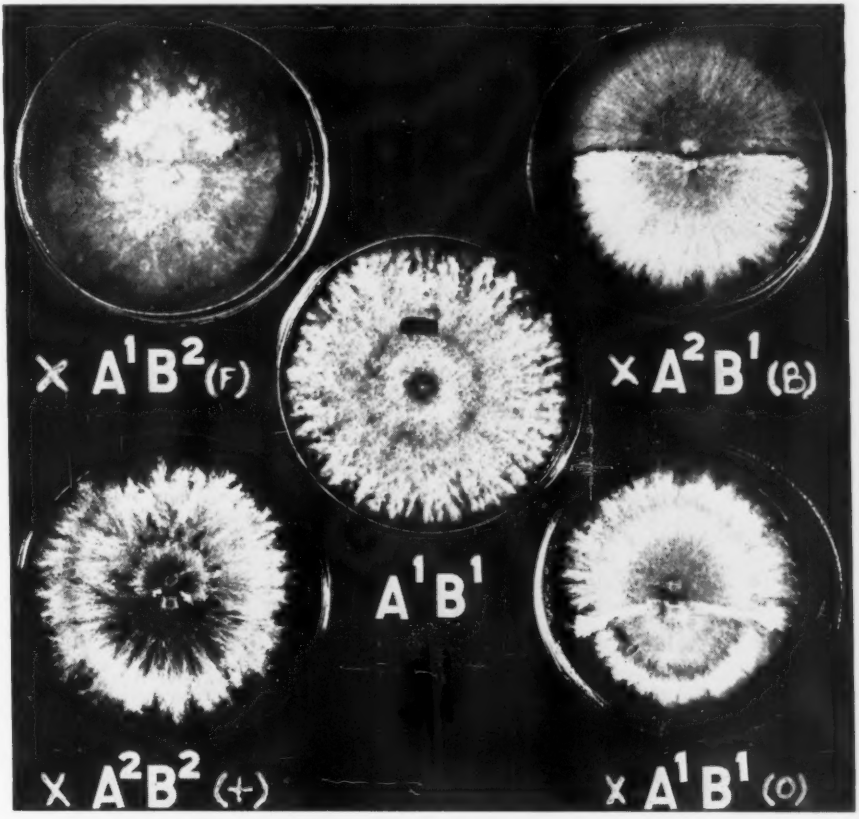


FIG. 1.—Mating reactions in *Schizophyllum*. A single monokaryon (A^1B^1) is shown in center. At four corners it is shown in matings with various other monokaryons. (F) = "flat," (B) = "barrage," (+) = dikaryon, (O) = overlap.

"flat," but the significance of their presence in these experiments is not known.

COMMON *A* AND *B* FACTORS.—A region of overlap where the two mycelia meet usually occurs when a mating is made between two mycelia having identical alleles at both the incompatibility loci (fig. 1). This pattern is, however, very irregular and may be expressed in varying degree. The incidence of overlap does not seem to be correlated with any particular incompatibility alleles. If one can make a comparison with the ascomycetes where heterokaryons are commonly formed between mycelia of the same mating type, it might be expected that in such matings there would be a mutual migration of nuclei from one mycelium into the other. No conclusive evidence is yet available to indicate whether this is the case, but one indication to the contrary follows

from the fact that, when there are recognizable differences in the morphology of the two monokaryons, these differences persist until both mycelia have grown to the edge of the Petri dish. As will be demonstrated below, most morphological characteristics of any mycelium are probably under very direct nuclear control.

DIFFERENT *A* AND *B* FACTORS.—A dikaryon results when monokaryons with different alleles at the *A* and *B* loci are mated. Nuclei from each mycelium migrate into the other, divide repeatedly, and form conjugate pairs with the original nuclei (3). The results of certain sur-

TABLE 1

	A^1B^1	A^2B^2	A^1B^2	A^2B^1
A^1B^1	O	+	F	B
A^2B^2	+	O	B	F
A^1B^2	F	B	O	+
A^2B^1	B	F	+	O

O = overlap; + = clamp connections; B = "barrage"; F = "flat." These symbols will be used throughout the paper.

gical operations on the dikaryon of *Schizophyllum* (12) indicate that either of the two nuclei can enter the clamp connection at any particular division. The dikaryotic mycelium is capable of fruiting, and in the present work every dikaryon, when grown in the special conditions favoring fruiting, gave rise to normal fruits, with the exception of a few dikaryons known to contain mutant genes.

Morphological differences between the two mated monokaryons do not persist after fusion of the mycelia. Although there is great variety in the morphology of various di[illegible]vons, the mycelia on both sides of [illegible] articular mating are nearly always identical. This indicates that the nucleus has direct control over the morphological characteristics of the dikaryon, a conclusion at slight variance with some of those reached by HARDER (12).

Pseudo-clamps were quite frequently observed in dikaryotic mycelia. They appeared more frequently in some mycelia than in others, but no correlation could be made between their appearance and any particular combination of incompatibility alleles. Pseudo-clamps and normal clamp connections both often appeared on the same hypha, and no evidence for a "clamp forming hormone" (12) was found.

Short sections of single hyphae, one to three cells long, when cut off and inoculated onto nutrient agar, gave rise to dikaryotic mycelia. Thus, of nineteen single hyphae of dikaryons, fourteen gave rise to normal dikaryons and five failed to grow.

Cytologically, long filaments were observed in all but the terminal cells or the terminal portions of long cells about to divide; small granules were observed in all cells. Both these types of inclusions were presumably mitochondria, but this was not confirmed. Although several nuclear divisions were followed, and the disappearance of the nuclear membrane and nucleolus was observed, spindle or chromosomes were never clearly apparent.

COMMON *B* FACTORS.—A "barrage" is formed in matings in which the *A* alleles are different and the *B* alleles are the same (fig. 1). This is a fortunate mnemonic association. The choice of symbols used here appears, moreover, to coincide, on the basis of the mutation rates of the two factors, with those used by KNIEP. The "barrage" region is not entirely devoid of hyphae, but these hyphae are thin, far apart, and rarely assume an aerial habit. Microscopically, hyphae in the "barrage" region are irregular, having numerous knobs and very short

branches. Subcultures from the "barrage" do not usually remain characteristic but revert to normal monokaryons, the mating types of which are those of the monokaryons used in the mating. The extent of the "barrage" region in different matings is very variable. It may be barely visible or it may be a centimeter or so wide. Occasionally whole sectors of mycelium resembling that found in the "barrage" region are found in matings with common *B* factors. In the particular case of a mating $A^1B^2 \times A^2B^2$, such a sector was observed on the A^2B^2 mycelium, and subcultures were made. Repeated subcultures from the "barrage" region of this mating exhibited a degree of stability. When mated with four tester monokaryons, they gave the following results:

	A^1B^1	A^2B^2	A^1B^2	A^2B^1
$A^1B^2 \times A^2B^2$	−/+	−/−	−/−	−/+

In this case, and throughout the paper, the reaction of the unknown mycelium is shown by a symbol to the left of the "/" sign and that of the tester to the right. A "−" sign indicates that no clamp connections or other recognizable reactions were found. The culture from $A^1B^2 \times A^2B^2$ did not have clamps in any mating but diploidized testers A^1B^1 and A^2B^1 unilaterally. It is concluded that this culture was a heterokaryon containing the two nuclei A^1B^2 and A^2B^2. It can be assumed that the hyphae in a narrow "barrage" region also constitute a heterokaryon but that it is generally very unstable and that the nuclei of each monokaryon do not generally migrate far into the opposing mycelium.

COMMON *A* FACTORS.—Matings of monokaryons having common *A* alleles show a characteristic pattern which appears with great regularity in all combinations of alleles and stocks tested. After the two mycelia have come into contact, further growth of both mycelia is thin, producing very few aerial hyphae. This type of growth has been named "flat." The general appearance of "flat" varies somewhat in matings of mycelia from different stocks but is generally similar on both sides of any particular mating. This similarity in the two mates following intermingling indicates, as in the dikaryon, that the morphology of the mycelium is under direct nuclear control.

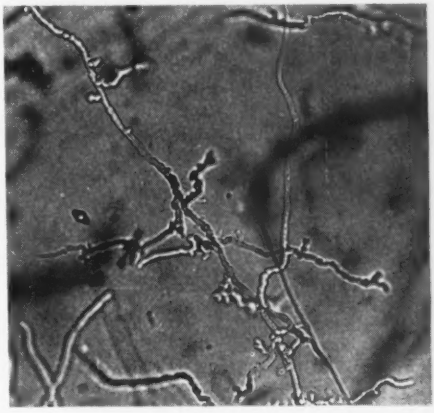


FIG. 2.—Hyphae of "flat" growing on agar

Microscopically, the hyphae of "flat" (fig. 2) resemble those found in a "barrage" region. The hyphae are narrow and have numerous knobs and fine branches. The number of nuclei per cell is variable. Most cells, including the terminal cells, have only one nucleus each, some cells have two or more nuclei, and a few cells are apparently devoid of nuclei. Where two nuclei were seen in the same cell, they were not in close association as are the nuclei of a dikaryon.

Subcultures taken from "flat" remain constant. When a "flat" culture is mated

with the four testers of its stock, the results are as follows:

	A^1B^1	A^2B^2	A^1B^2	A^2B^1
$A^1B^1 \times A^1B^2$	F/F	F/+	F/F	F/+

"Flat" unilaterally diploidizes monokaryons with which its two component nuclei are compatible. It has been found that unilateral diploidization is a constant characteristic of all "flat" mycelia. Subcultures of the testers A^2B^2 and A^2B^1, diploidized by "flat" in the above matings, fruited in every instance.

The failure to form clamp connections in "flat" mycelia when mated with compatible monokaryons does not absolutely preclude the migration of compatible nuclei into "flat." In order to test whether such migrations actually take place, a number of complex matings were performed, in duplicate, as follows:

$$[(A^1B^1 \times A^1B^2) \times A^2B^1] \times A^1B^2;$$
$$[(A^1B^1 \times A^1B^2) \times A^2B^1] \times A^1B^2;$$
$$[(A^1B^1 \times A^1B^2) \times A^2B^2] \times A^1B^1;$$
$$[(A^1B^1 \times A^1B^2) \times A^2B^2] \times A^1B^1.$$

In no case was there diploidization. From this it can be concluded that the nucleus of a compatible monokaryon does not migrate into "flat" or that, if a few nuclei do migrate, they do not multiply.

Matings were also made between "flat" and cultures of the "barrage" sector described in the previous section. In no case was there diploidization of either heterokaryotic mycelium.

Single hyphae of several "flat" mycelia were cut off and cultured. In only one case out of twenty was the resulting mycelium "flat"; in the nineteen other cases the appearance of the mycelium was that of a normal monokaryon, and the mating type of each was that of one or the other parental mycelium. In the exceptional case, diploidization was unilateral as it is in "flat," but the dual mating type characteristic of "flat" was not exhibited.

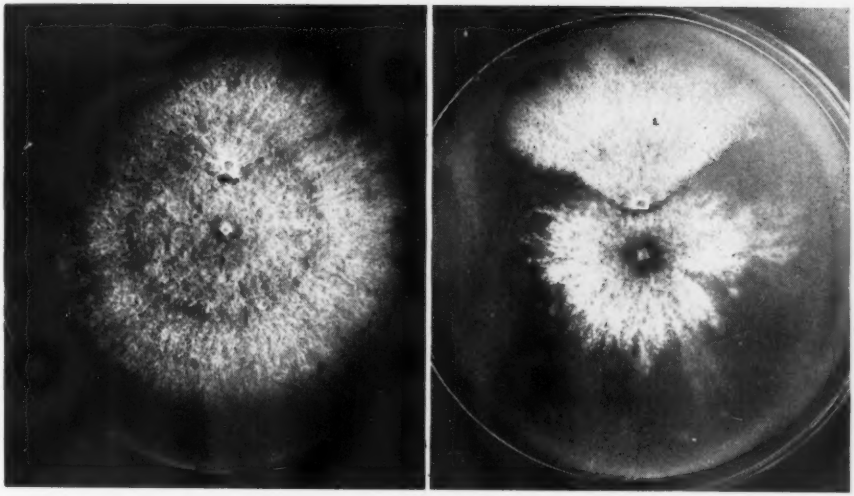
The general conclusion is that "flat" is a heterokaryon but that not all cells, or even most cells, contain both types of nucleus.

Di-mon matings (the "Buller phenomenon")

In hemi-compatible di-mon matings of the general type $(A^1B^1 + A^2B^2) \times A^1B^1$ it has been found, with few exceptions, that the monokaryon is diploidized evenly and rapidly throughout its whole extent (fig. 3).

In noncompatible di-mon matings of the general type $(A^1B^1 + A^2B^2) \times A^1B^2$, on the contrary, the monokaryon is diploidized in only about half of the cases and, even then, only in patches. In noncompatible di-mon matings a "barrage" is often formed at the junction of the two mycelia, and, characteristically, the subsequent growth, on the monokaryon side of the mating, has the appearance of "flat" (fig. 4). Subcultures of this apparently "flat" growth remain constant in appearance. When mated with testers, they behave like a "flat" mycelium containing a nucleus of the mating type of the monokaryon and another of the mating type of one of the nuclei associated in the dikaryon. In the example given above the A^2B^2 nucleus of the dikaryon probably forms a limited "barrage" heterokaryon with the monokaryon (A^1B^2), and the A^1B^1 nucleus sets up a stable "flat" heterokaryon.

The occurrence of diploidization in noncompatible di-mon matings is much more frequent in some combinations than in others (table 2). The number of matings made is too small for any cor-



FIGS. 3, 4.—Fig. 3 (*left*), hemi-compatible di-mon mating. Fig. 4 (*right*), noncompatible di-mon mating. Dikaryon was inoculated above monokaryon in each case.

TABLE 2

NONCOMPATIBLE DI-MON MATINGS

Constitution of mating	No. of matings made	No. in which diploidization occurred	No. analyzed	Constitution of dikaryon	Interpretation*
$(A^1B^1+A^2B^2)\times A^1B^3$	4	2	0	.....	.....
$(A^1B^1+A^2B^2)\times A^2B^1$	4	1	0	.....	.....
$(A^1B^2+A^2B^1)\times A^1B^1$	5	2	2	$A^1B^2+A^2B^1$	A
$(A^1B^2+A^2B^1)\times A^1B^1$	4	1	1	$A^1B^2+A^2B^1$	A
$(A^1B^2+A^2B^1)\times A^2B^2$	6	1	1	$A^1B^2+A^2B^1$	A
$(A^1B^3+A^3B^1)\times A^1B^1$	2	1	0	.....	.....
$(A^1B^3+A^3B^1)\times A^3B^3$	4	4	4	$A^1B^1+A^3B^3$	B
$(A^1B^3+A^3B^1)\times A^3B^3$	2	2	2	$A^1B^1+A^3B^3$	B
$(A^3B^4+A^4B^3)\times A^4B^4$	2	0	.....	.....	.....
$(A^3B^4+A^4B^3)\times A^3B^3$	2	2	2	$A^3B^4+A^4B^3$	A
$(A^3B^3+A^4B^4)\times A^3B^4$	1	1	1	$A^3B^3+A^4B^4$	A
$(A^3B^3+A^4B^4)\times A^4B^3$	1	1	1	$A^3B^3+A^4B^4$	A
$(A^1B^1+A^3B^3)\times A^1B^3$	2	2	2	? + ?	.....

* A, migration of both nuclei of dikaryon into monokaryon. B, constitution of new nucleus from two nuclei of dikaryon.

relations to be made with the various alleles and stocks.

The mechanism by which the monokaryon is diploidized in a noncompatible di-mon mating has aroused considerable interest and speculation. Three possibilities have been considered (**24**): (*a*) A mutation, occurring in one of the incompatibility alleles, renders the nucleus of the monokaryon compatible with one of the nuclei of the dikaryon. (*b*) Both nuclei of the dikaryon, migrating into the monokaryon, displace the nuclei of the latter in subsequent growth—this hypothesis was supported by RAWITSCHER (**27**), BULLER (**4, 5**), CHOW (**6**), DICKSON (**8**), and OIKAWA (**21**) but lacks clear experimental basis. (*c*) A new nucleus, e.g., A^1B^2, is formed from the two nuclei of the dikaryon, e.g., A^1B^1 and A^2B^2. This last hypothesis is supported by some experimental work of QUINTANILHA (**24**).

TABLE 3

ANALYSIS OF DIPLOIDIZED MONOKARYON FROM $(A^1B^1 + A^2B^2) \times A^1B^2$

	A^1B^1	A^2B^2	A^1B^2	A^2B^1
$A^1B^1 + A^2B^2$	H	H	N	N
$A^1B^2 + A^2B^1$	N	N	H	H
$aB^1 + A^1B^2$	N	H	H	H

H = Hemi-compatible di-mon; N = Noncompatible di-mon.

When the striking difference in appearance between hemi-compatible and noncompatible di-mon matings was observed, it was evident that here was the basis for a technique by means of which the constitution of any given dikaryon could be readily determined. Only one attempt to determine the constitution of a dikaryon experimentally had hitherto been reported (**24**), and the method was laborious, involving the production of fruiting-bodies. A new technique was developed, therefore, to determine the nuclear constitution of the dikaryon formed in a noncompatible di-mon mating and obtain evidence as to which of the three possibilities listed above occurred in any particular case. The constitution of the dikaryon formed by the noncompatible di-mon $(A^1B^1 + A^2B^2) \times A^1B^2$, for instance, might be either $A^1B^1 + A^2B^2$, or $A^1B^2 + A^2B^1$, or might include a mutant such as $aB^1 + A^1B^2$. If this unknown dikaryon were mated with four appropriate testers, these three possibilities could be distinguished (table 3).

Sixteen successful noncompatible di-mon matings were tested in this way. It was found that more definitive results were obtained if the unknown dikaryon were subcultured at least once before testing and if the first inoculum were taken from the periphery of the monokaryon side of the di-mon mating most remote from the dikaryon. Another routine procedure was to inoculate the tester monokaryon 24 hours before the unknown dikaryon in each test. The results are shown in table 2. In fourteen cases the results were unambiguous; the other two, a set of duplicates, gave anomalous results.

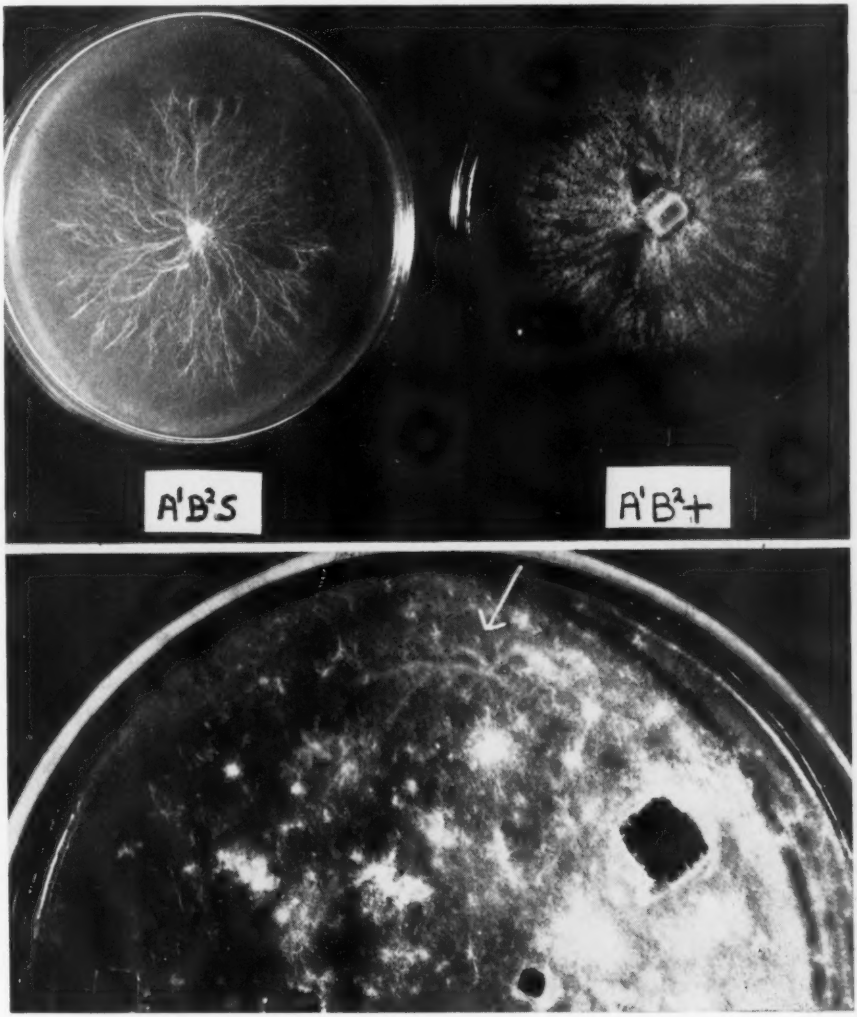
In five of the six different combinations represented among the unambiguous cases both nuclei of the dikaryon migrated into the monokaryon; in the sixth case a new nucleus was constituted. This was true in all six replicates of this mating, irrespective of the side of the first mating from which the inoculum for the di-mon mating was taken.

"STREAK"

A characteristic type of growth originates, under certain conditions, from "flat" mycelia or from combinations of monokaryotic mycelia which would produce "flat." This atypical form remains constant on subculturing, is easily distinguished macroscopically (fig. 5), and

has several interesting properties. This type of mycelium, owing to its physical appearance, has been named "streak" ("s").

"Streak" once originated in a mating of a tester monokaryon A^2B^2, with a mycelium derived from a single hypha of the "flat" $A^1B^1 \times A^1B^2$. The single-hypha mycelium had the mating type A^1B^2 and was the particular one mentioned above in the section "Common *A* factors." The behavior of this mycelium was anomalous in that diploidization was unilateral. The phenomenon of "streak" origin was



FIGS. 5, 6.—Fig. 5 (*above*), "streak" (*left*) and wild-type (*right*) monokaryons. Fig. 6 (*below*), origin of "streak" from a blended "flat" culture.

also seen several times in complex crosses involving "flat" such as $[(A^1B^1 \times A^1B^2) \times A^2B^2] \times A^1B^1$. In these cases the "streak" appeared as a sector in the "flat."

The production of "streak" is favored by blending the appropriate mycelium or mycelia in a Waring Blendor. The two monokaryotic mycelia can either be grown in liquid culture separately and then blended together, or a "flat" mycelium can be grown in liquid culture and this single mycelium blended. In either case the suspension that results from the blending is plated out thinly on nutrient agar and allowed to incubate at room temperature. In these cases which involve blending, the "streak" only appears after the parent culture is very old. It appears, moreover, not as a sector, but as a new growth originating independently at several centers distributed randomly throughout the plate (fig. 6). When a suspension of a blend is plated out, at least one growth of "streak" is produced in every resulting culture.

"Streak" is distinguished microscopically by the coarse, ropy appearance of the mycelium (figs. 5, 7). Generally there are few aerial hyphae, although in old, dry cultures very long aerial hyphae are often produced in localized regions of the mycelium. The growth rate of "streak" is about the same as that of a wild-type monokaryon. The ropy appearance is probably due both to the rather numerous branches on the hyphae and to the pattern of branching. The branches are short, tend to occur in whorls, and usually make an acute angle with the main hypha.

Most cells of "streak" appear to have a single nucleus, although two or more nuclei have been seen in some cells. The hyphae are thick, being of a diameter equal to or greater than the hyphae of a wild-type monokaryon.

"Streak" behaves as a wild-type monokaryon in all but one important respect in matings. It always diploidizes unilaterally, no "streak" mycelium ever having been found to form clamp connections. "Streak" forms a "flat" mycelium with a monokaryon having a similar *A* factor, just as any wild-type monokaryon does, and as "flat" itself does. The process, in the case of "streak," however, is unilateral, and the "streak" mycelium remains unaltered in appearance. The mating type of "streak" is always that of one or the other of the component mycelia from which it originated. Limited analysis of three independent cultures proved that "streaks" of both mating types occur in the same culture. The "streak" character is recessive to wild-type in the sense that dikaryons and "flat" mycelia containing a "streak" and a wild-type nucleus resemble the wild-type in appearance.

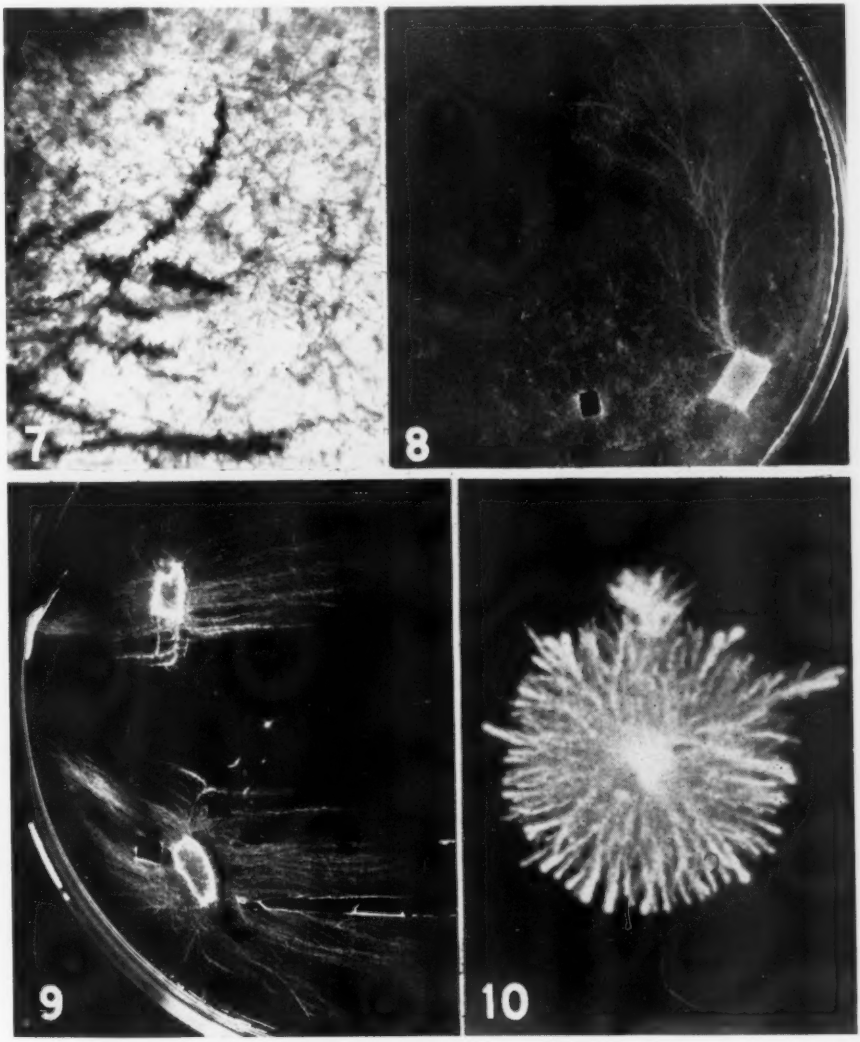
A "flat" mycelium containing a "streak" nucleus can be subcultured. "Streak" sectors appear, however, very frequently in such subcultures (fig. 8). Characteristic "streak" mycelia result in practically all cases from the transplantation of single hyphae, or even of small inocula containing about one hundred cells, taken from "flat" mycelia having a "streak" nucleus. Thus, seventeen of twenty small inocula from a "flat" produced by mating a "streak" of mating type A^1B^1 with a wild-type monokaryon of mating type A^1B^2, i.e., $A^1B^1s \times A^1B^2+$, gave rise to "streaks"; two others were wild-type, and one did not grow. The seventeen "streaks" were all A^1B^1s; the wild-type were A^1B^2+.

"Streak" occasionally sectors out of a dikaryon having a "streak" nucleus but not so frequently as it does from a "flat"

heterokaryon. Similarly, nearly all single hyphae from such dikaryons grow into dikaryons and only rarely into "streak" mycelia.

The following preliminary experiments were made to ascertain some of the factors that might be involved in the regular production of "streak."

"Streak" mycelia can be isolated from the conglomerate culture produced when a small amount of "streak," e.g., A^1B^1s, is blended with the monokaryons A^1B^1+



FIGS. 7–10.—Fig. 7, hyphae of new "streak" growth on old culture of blended "flat." Fig. 8, "streak" sector in "flat" mycelium containing "streak" nucleus ($A^1B^1s \times A^1B^2+$). Fig. 9, "streak" mycelium showing phenomenon of elasticotropism. Fig. 10, dwarf "streak."

and A^1B^2+ and the resulting mixture is plated out. The appearance of "streak" growth in a conglomerate culture in which "streak" nuclei have been deliberately added is different from that which appears in a similar culture in which no such nuclei are introduced. When these nuclei are added, growths of "streak" appear in very young cultures, most frequently as sectors around the periphery of the growing culture.

Blends of single monokaryons do not give rise to "streak." The question of the behavior of a "streak" nucleus in a mycelium of its own mating type has not yet been resolved. It would appear, moreover, that the nuclei do not migrate in a mating such as $A^1B^1s \times A^1B^1+$, since all efforts to obtain "streak" from the wild-type partner in such matings have failed.

Experiments were performed to find out whether nuclei could migrate into a "streak" mycelium. Complex matings similar to those described in connection with "flat" were used. It was found that both in matings producing "flat" and in matings producing dikaryons, the wild-type nucleus failed to migrate into the "streak" mycelium.

Another characteristic of "streak" mycelia is that they never fruit. Many combinations were repeatedly but unsuccessfully put to fruit.

Two further properties distinguish "streak" from wild-type mycelia: First, "streak" produces two distinct pigments: a pale, yellow, water-soluble pigment occurring sporadically in the mycelium itself, especially in old cultures, and an intense, greenish-blue pigment, very striking in appearance, forming in the medium in very old cultures, especially in those stored for some time at 5° C. Second, "streak" mycelia display "elasticotropism," an imaginative but accepted term which fits the present data. Elasticotropism denotes a directed growth along the lines of stress in the medium. It has been observed and studied in various bacteria and has been shown to be of significance in embryology (**30**). It has not, so far as the author is aware, been reported in any plant except the bacteria. This phenomenon is most easily demonstrated by transplanting "streak" onto the center of the agar in a Petri dish and making a long cut in the agar almost to the sides of the dish. If the agar is moderately dry, the cut gapes open as a result of shrinkage of the agar, and tension is set up in the agar parallel to the cut (fig. 9). Groups of hyphae orientated at right angles to the cut divide into two smaller groups which proceed to grow in opposite directions parallel to the cut. The phenomenon of elasticotropism can also be demonstrated by growing "streak" on a small rectangular block of agar laid on a glass rod to produce a strain as in the experiments of STANIER (**28**). The strain can be set up along the long, or the short, axis of the rectangular block, and in either case growth of the "streak" mycelium is in a direction parallel to the strain. In a further experiment a strain was set up in agar by pouring a small amount of agar onto a strip of sheet rubber, carefully stretching the strip of rubber after the agar had set and had partially dried, and anchoring the strip in the stretched position. Again growth was along the lines of stress in the agar.

KUFFERAT (**18**) found that elasticotropism was exhibited by *Bacterium zopfii* only when gelatin was used in the medium. There was no such effect with agar. In experiments with "streak" the opposite was found to be true, the effect being exhibited on agar but not on gelatin,

since gelatin is liquefied by "streak." Because dializing sheet cellophane is anisotropic, it was thought that the phenomenon of elasticotropism might be induced by it. Squares of cellophane were placed on nutrient agar and centrally inoculated with "streak." In no instance was any tropic growth observed in the mycelia which grew out from the inocula.

"STREAK" COMPARED WITH OTHER TYPES OF MYCELIA.—The growth rate of "streak" on basic nutrient medium is about the same as that of the wild-type monokaryon and that of the dikaryon.

When the medium was adjusted from pH 6.3 to 6.8, the four different types of mycelia—"flat," "streak," wild-type monokaryon, and dikaryon—grew well. The growth was similar in appearance to that on basic medium with the exception of the monokaryon and dikaryon, each of which developed less than the normal quantity of aerial hyphae. At pH 3.8 "flat," monokaryon, and dikaryon grew more slowly, and "streak" grew much more slowly than on basic medium. The difference in growth rates between "streak" and the other mycelia at the two pH levels was striking. The "streak" mycelium, furthermore, did not exceed a diameter of about 3 cm. in the acid media. All four mycelia, including "streak," produced well-developed aerial systems at pH 3.8.

The effects of lowered oxygen tension were determined. Cultures of the four types of mycelia, growing on basic medium, were placed under a bell jar in which the air was displaced by nitrogen. Under these semianaerobic conditions growth of all four mycelia was retarded, and there was in each case a marked absence of aerial hyphae. No new differences between the different types of mycelia were observed; in fact, they resembled one another more than when grown under standard conditions. On subculturing to standard aerobic conditions they all grew normally.

On inorganic medium containing glucose, asparagine, and pyrimidine the four mycelia, including "streak," appeared normal but grew slowly. A nitrite-mannitol medium (29) killed all four mycelia. When this medium was diluted by an equal amount of basic medium, all four mycelia grew slowly, and neither the monokaryon nor the dikaryon produced aerial hyphae. Subcultures onto basic medium were all normal, and no mutants were observed.

VARIETIES OF "STREAK."—Most of the work described above was done with cultures derived from I (arg). The main results were, however, checked with stocks from XV (ad) and XXV (mad), and it is probable that they could be duplicated in most stocks of the species. The various "streak" cultures derived, as previously described, from the three stocks agreed for the most part with the description given above. Some aberrant cultures were obtained, however, and they differed in several respects. The most frequent aberrant type was a dwarf "streak" (fig. 10). Only one of these was tested, and it exhibited the dual mating type characteristic of "flat" when mated with testers of its own stock but was incompatible with testers of the other two stocks. Two other aberrant "streaks" inhibited the growth of all testers with which they were mated and only diploidized testers of stocks different from their own. All the aberrant "streaks" resembled typical "streaks" in diploidizing unilaterally. These, and other, aberrant "streaks" will be reported on more fully as further data become available.

The origin, properties, and mating

characteristics of the four types of mycelia are summarized in table 4.

Compatible di-mon matings

In *Coprinus fimetarius*, Quintanilha (**26**) found that, in a situation such that a monokaryon could be diploidized by two or more different nuclei simultaneously, the particular nucleus diploidizing the monokaryon was not a matter of chance but was always the same. Two series of experiments were performed. In one series three or four different monokaryons were inoculated on the same plate, and the constitution of the resulting dikaryon was determined; in the other series compatible di-mon matings were made, and the constitution of the newly formed dikaryon was determined. In these latter experiments it was found that the particular side of the original mating which was subcultured for the di-mon mating was irrelevant to the constitution of the resulting dikaryon. Thus $(A^1B^1 + A^2B^2) \times A^3B^3$ gave rise to the same dikaryon as $(A^1B^1 + A^2B^2) \times A^3B^3$.

Quintanilha also found that the constitution of the resulting dikaryon was dependent on the particular A alleles involved and that the A alleles could be arranged in a linear order of *strength* such that, where a choice existed, those nuclei having A alleles more than a certain value apart always formed the dikaryon.

In view of the interesting results reported by Quintanilha, experiments were made to determine whether a similar pattern could be demonstrated in *Schizophyllum*. Compatible di-mon matings were employed in this series. The constitution of the resulting dikaryon was determined by further di-mon matings as described in the section "Di-mon matings (the 'Buller phenomenon')." For the di-mon $(A^1B^1 + A^2B^2) \times A^3B^3$, for example, the testers A^1B^3, A^3B^1, A^2B^3, and A^3B^2 were used. These would distinguish the constitutions $A^1B^1 + A^3B^3$, $A^2B^2 + A^3B^3$, $A^1B^1 + A^2B^2$, $A^1B^2 + A^3B^3$, and $A^2B^1 + A^3B^3$. For other possible constitutions other testers would be required.

Results of the analysis of nineteen different combinations are shown in table 5. Five additional combinations persistently gave results which cannot be interpreted without making complex and improbable assumptions, and they have been omitted from the table.

Formulation of a simple rule that

TABLE 4

Properties of wild-type monokaryon, "flat," "streak," and dikaryon

	Wild-type single-spore mycelium	Flat	Streak	Dikaryon
Origin	A^1B^1 or A^1B^2 mated → $A^1B^1 \times A^1B^2$		blended → A^1B^1 or A^1B^2	$A^1B^1 + A^2B^2$
Type of mycelium	Monokaryon	Heterokaryon	Monokaryon	Dikaryon
Number of nuclei per cell	One	Usually one	Usually one	Two
Mating reaction	Single	Dual	Single	Dual*
Diploidization	Bilateral	Unilateral	Unilateral	.....
Elasticotropism	.....	(+)†	+	.....
Pigment	None	None	Yellow and dark blue	None

* In di-mon matings.

† An occasional, barely perceptible, reaction.

would account for these results is not easy or attempted here. The side of the original mating from which the inoculum for the compatible di-mon mating is taken was in every case irrevelant to the constitution of the resulting dikaryon. The choice of nuclei appears to be definitely governed by some factor other than the alleles of either of the incompatibility factors. The situation is thus quite different from that in *C. fimetarius*. It should be emphasized that, although all the monokaryons of the same mating type used in this experiment came from the same culture, nothing is known of their genetic constitution apart from the incompatibility alleles. The experiment would yield more valuable results if isogenic strains were used.

Two interesting facts emerge from the results. In two combinations, both nuclei of the dikaryon apparently migrated into the monokaryon and became established there. This is what QUINTANILHA (24) found in one exceptional mating. It was also a compatible di-mon mating. More unexpected are the five different combinations in which new nuclei were constituted. This is the first record, to the author's knowledge, of the apparently useless occurrence of such a process in compatible di-mon matings.

TABLE 5

COMPATIBLE DI-MON MATINGS

Compatible di-mon mating	Constitution of resulting dikaryon	No. of times performed	Remarks
$(A^1B^1+A^2B^2)\times A^3B^3$	$A^2B^2+A^3B^3$	3	
$(A^1B^1+A^2B^2)\times A^3B^3$	$A^2B^3+A^3B^3$	2	
$(A^1B^1+A^2B^2)\times A^3B^4$	$A^2B^2+A^3B^4$	1	
$(A^1B^1+A^2B^2)\times A^4B^3$	$A^2B^1+A^4B^3$	1	New nucleus
$(A^1B^1+A^2B^2)\times A^4B^3$	$A^2B^1+A^4B^3$	1	New nucleus
$(A^1B^2+A^2B^1)\times A^4B^3$	$A^2B^2+A^4B^3$	1	New nucleus
$(A^1B^1+A^2B^2)\times A^4B^4$	$A^1B^1+A^4B^4$	1	
$(A^1B^2+A^2B^1)\times A^4B^4$	$A^1B^2+A^4B^4$	1	
$(A^1B^1+A^3B^3)\times A^2B^2$	$A^3B^3+A^2B^2$	1	
$(A^1B^1+A^4B^4)\times A^2B^2$	$A^4B^4+A^2B^2$	1	
$(A^1B^1+A^4B^4)\times A^2B^2$	$A^4B^4+A^2B^2$		
$(A^1B^1+A^3B^4)\times A^3B^3$	$A^1B^1+A^4B^4$ or $A^1B^4+A^4B^1$	1	Both nuclei migrated
$(A^1B^1+A^4B^4)\times A^3B^2$	$A^1B^1+A^3B^2$	1	
$(A^2B^2+A^3B^3)\times A^4B^4$	$A^3B^3+A^4B^4$	1	
$(A^2B^3+A^3B^2)\times A^4B^4$	$A^2B^2+A^4B^4$	1	New nucleus
$(A^1B^3+A^3B^2)\times A^4B^1$	$A^2B^3+A^4B^1$	1	
$(A^2B^3+A^3B^2)\times A^1B^4$	$A^2B^2+A^1B^4$	1	New nucleus
$(A^2B^3+A^3B^2)\times A^1B^1$	$A^2B^2+A^1B^1$	1	New nucleus
$(A^1B^3+A^4B^4)\times A^1B^1$	$A^1B^3+A^4B^4$ or $A^3B^4+A^4B^3$	1	Both nuclei migrated

Discussion

The different patterns of reactions found in matings between various types of monokaryotic mycelia in *S. commune* are not restricted to this species, and they may well be of widespread occurrence. It has been reported in various species of hymenomycetes that a "barrage" is formed when two monokaryons having

alleles of a particular factor in common are mated (1). An intensive study of the literature has revealed that both "barrage" formation and a reaction very similar to "flat" have been reported in *Pleurotus corticatus* (14) and that a pattern of behavior very similar to that reported here in noncompatible di-mon matings has been described in *Pleurotus ostreatus* (15). In the gasteromycete *Cyathus*, FULTON (10) has shown that there are three distinct patterns obtained in matings between monokaryotic mycelia and that these correspond in some details to the patterns found in *Schizophyllum*. It is difficult to assess how closely the phenomena described in these various species resemble the present findings in *S. commune*. The present work has demonstrated that "flat" is a rather stable heterokaryon and that the mycelium in a "barrage" region, in some cases, if not in all, is also heterokaryotic, having properties very similar to those of "flat." Comparing this situation with the descriptions of "barrages" in other organisms, it appears that in no case has the formation of a "barrage" been attributed to heterokaryosis and that in the case of *Lenzites betulina*, according to published descriptions (1), the formation of a heterokaryon seems very unlikely. As regards "flat," the response of certain monokaryons in *P. corticatus*, as far as one can judge from the available description, fits very well with that giving rise to "flat" mycelia in *S. commune*, and it is unfortunate that the phenomenon was not more intensively studied in that species. The peculiar type of growth which was observed in noncompatible di-mon matings in *P. ostreatus*, on the other hand, did not persist in subcultures and would appear to differ in this and in other respects from the "flat" of *S. commune*. KAWAMURA concluded that this peculiar type of growth in *P. ostreatus* is due to some reversible cytoplasmic effect.

The demonstration, in *Schizophyllum*, that heterokaryons are produced in matings between incompatible mycelia is significant, because it indicates that incompatibility does not depend on failure of hyphal fusion or even on failure of nuclear migration from one mycelium into the other. The incompatibility genes must be effective at some subsequent stage in the series of processes that lead to fruiting. The stable heterokaryon "flat," which results from incompatible matings, can be compared with the "balanced heterokaryons" described in other organisms by PONTECORVO (23). The fundamental difference between "flat" and these "balanced heterokaryons" is that, in the case of "flat," there is no obvious selective mechanism that would account for the stability of the heterokaryon. It must therefore be assumed that the rate of division of each type of nucleus is controlled by some unknown factor in such a way that the numbers of the two types of nuclei remain approximately equal.

One of the most interesting features of "flat" and of cultures from "barrage" regions is the property of unilateral diploidization. This property is not unique in *S. commune* among the basidiomycetes. GREIS (11) has described the regular occurrence of unilateral diploidization in matings between monokaryons of the hymenomycete, *Solenia anomala*. BRODIE, working with the gasteromycete *Cyathus stercoreus*, described various conditions intermediate between perfect bilateral diploidization and perfect unilateral diploidization in the paper (2) in which the term "unilateral diploidization" was originally used. FULTON (10), using the same species as BRODIE, found that unilateral diploidization depended

upon the "acceptor" mycelium, not on the "donor" mycelium, and that the "acceptor" mycelium had two or more nuclei per cell. Such mycelia were not, however, heterokaryons comparable with "flat." Exceptional cultures have been described in *Coprinus sphaerosporus* (8) and *C. fimetarius* (25), which diploidize unilaterally.

Heterokaryosis is not the only condition which gives rise to unilateral diploidization. The monokaryon "streak" also diploidizes unilaterally. It will be shown in a further publication that "streak" is a single-gene mutant. The regular occurrence of "streak" under appropriate conditions is therefore of great interest, and investigations are in progress on the nature of the processes involved.

The present data support the theory of QUINTANILHA (26) that a new nucleus is sometimes formed in noncompatible di-mon matings. There is also evidence from these data that a new nucleus is sometimes formed in compatible di-mon matings, though the manner in which this is effected remains obscure. The present data favor neither of the two most likely hypotheses, viz., somatic interchange of chromosomes or fusion of nuclei followed by reduction. By obtaining genetic markers on each of the three chromosomes, this problem can be resolved.

It is of interest that the effective combination of nuclei in compatible di-mon matings is not random but that in a particular cross the same nucleus of the established dikaryon always diploidizes the monokaryon. This suggests that the different alleles are not completely equivalent but that there is a selection for given alleles under certain circumstances. That nonequivalence of the incompatibility alleles holds in some other organisms is indicated by the replacement of one type of nucleus in a mycelium by another reported by DICKSON (9), the relativity phenomenon described by LORENZ (20), and the experiments of QUINTANILHA described above. Mutations of incompatibility alleles are of frequent occurrence in *Schizophyllum* and will be described in a separate paper. A selection for certain alleles in a population was demonstrated by KNIEP (17), who grew twenty-four generations of *S. commune* by making mass spore cultures of each generation to obtain the fruiting-body of the next generation. For the first nine generations the allelic composition of the spores was variable, but all subsequent generations had the same four incompatibility alleles, two of these having been among those present in the original culture and two being new.

The finding that heterokaryons are formed in various incompatible matings and the discovery of the mutant "streak" which diploidizes unilaterally provide a basis on which some theory of the structure of the incompatibility factors can be laid. They open the field to varied future investigations which may prove the basidiomycetes to rank high among the organisms used for studies in physiological genetics.

Summary

1. A standard system of nomenclature is used and is recommended for general adoption. This system proposes the following terms: *monokaryon*, the monosporous mycelium containing only a single type of nucleus; *dikaryon*, the "secondary mycelium" containing conjugate nuclei; *A* and *B*, specifically identified by superscripts, incompatibility factors at the two loci; *di-mon* matings, matings involving diploidization of a monokaryon by a dikaryon.

2. Four distinct types of reaction are obtained in matings between monokaryons of any stock. These reactions depend on the identity or difference of the alleles at each locus, thus:

$A^1B^1 \times A^2B^2$—dikaryon
$A^1B^1 \times A^1B^1$—overlap
$A^1B^1 \times A^2B^1$—"barrage"
$A^1B^1 \times A^1B^2$—"flat"

3. "Flat," and, in some instances, "barrage," can be subcultured. Each exhibits a dual mating type, diploidizing the two types of monokaryon that their parental mycelia diploidize. From this and other observations it is concluded that "flat" and "barrage" are heterokaryons. Only a minority of the cells of "flat," however, contain more than one nucleus. "Flat" and "barrage" always diploidize unilaterally and have never been found to produce clamp connections themselves.

4. Three types of di-mon matings are distinguished: (*a*) Hemi-compatible, i.e., $(A^1B^1 \times A^2B^2) \times A^1B^1$, in which one of the nuclei of the dikaryon is compatible with that of the monokaryon ("legitimate"); (*b*) noncompatible, i.e., $(A^1B^1 \times A^2B^2) \times A^1B^2$, in which neither is compatible ("illegitimate"); and (*c*) compatible, i.e., $(A^1B^1 \times A^2B^2) \times A^3B^3$, in which both are compatible.

5. Diploidization is much more regular in hemi-compatible than in noncompatible di-mon matings. The constitution of the diploidized monokaryon in a noncompatible di-mon mating was determined by a new method, and it was found that a new nucleus is sometimes formed in these matings.

6. An aberrant type of monokaryon, "streak," is described. "Streak" shows many peculiar properties and resembles "flat" in diploidizing unilaterally in all compatible matings.

7. "Streak" mycelia are regularly and uniquely obtained from mixtures of two monokaryons having common *A* factors, i.e., from "flats."

8. A series of compatible di-mon matings were made with mycelia having various incompatibility alleles, and the type of the nucleus that diploidized the monokaryon in each case was determined. No relativity phenomena comparable with those described by QUINTANILHA were found, but certain regular departures from random diploidization were observed. In some cases a new nucleus was superfluously formed; in others, both nuclei of the dikaryon migrated into the monokaryon, displacing its nuclei.

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GENE ANALYSIS OF WEIGHT PER LOCULE IN TOMATO HYBRIDS

LEROY POWERS[1]

Introduction

The scales of measurement appropriate for studying weight per locule in the parental and hybrid populations of the tomato crosses Danmark × Johannisfeuer, Danmark × Red Currant, and Johannisfeuer × Red Currant have been determined (**14**). The present article reports on a gene analysis of weight per locule, for the above hybrids, based on the scales of measurement found to be appropriate for describing the biological processes bringing about the expression of the character.

Materials and experimental design

The parents involved were Danmark (*Lycopersicon esculentum* Mill.), Red Currant (*L. pimpinellifolium* Mill.) and Johannisfeuer (*L. esculentum* Mill.). The statistical design of the experiment was a randomized complete block with twenty

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blocks, two plots of each segregating generation being grown per block to one plot of the two parents and F_1 in 1938, and one plot of each population being grown per block in 1939. In 1938 twenty-four plants were grown per plot and in 1939 only twelve. The genetic design of the experiment included every possible different hybrid between the three parents and the P_1, B_1 to P_1, F_1, F_2, B_1 to P_2, and P_2 populations for each hybrid. The parental and hybrid populations of Danmark × Johannisfeuer and Danmark × Red Currant were grown at the Horticultural Field Station, Cheyenne, Wyoming, in 1938, and those of Johannisfeuer × Red Currant were grown at the same location in 1939. The partitioning method (15) was used to analyze the data.

TABLE 1

DEGREES OF FREEDOM, CHI-SQUARE VALUES, AND *P* VALUES FOR TESTING GOODNESS OF FIT BETWEEN OBTAINED FREQUENCY DISTRIBUTIONS AND THOSE CALCULATED ON BASES OF ARITHMETIC AND OF LOGARITHMIC SCALES FOR WEIGHT PER LOCULE OF FRUIT; DANMARK × JOHANNISFEUER TOMATO HYBRID GROWN IN 1938

Population	Degrees of freedom		Chi square		*P* lies between	
	Arithmetic	Logarithmic	Arithmetic	Logarithmic	Arithmetic	Logarithmic
Johannisfeuer	8	10	11.636	44.326	0.20 & 0.10	0.01 & ....
B_1 to Johannisfeuer	13	15	118.466	35.936	.01 &	.01 & ....
F_1	14	13	67.582	6.800	.01 &	.95 & 0.90
F_2	16	19	136.745	33.310	.01 &	.02 & .01
B_1 to Danmark	16	17	77.482	47.078	.01 &	.01 & ....
Danmark	16	16	52.572	17.016	0.01 &	0.50 & 0.30

Results

DANMARK × JOHANNISFEUER

In a previous publication on determining scales and the use of transformations POWERS (14) has shown that for the 1938 data the environmental variability for the Johannisfeuer population followed the arithmetic scale and that those for the F_1 and Danmark populations followed the logarithmic scale. The environmental variability of the segregating populations (B_1 to Johannisfeuer, F_2, and B_1 to Danmark) followed neither scale, arithmetic or logarithmic. The data were interpreted as showing that all the segregating generations possessed plants of two types as regards scales of measurement appropriate for describing the environmental variability. Since the environmental variability of Johannisfeuer plants followed the arithmetic scale and that of the F_1 and Danmark plants the logarithmic scale, such might be expected. The data are presented in table 1.

The data in table 1 provide the information necessary for determining the scales of measurement appropriate for describing the environmental variability of the different genotypes of the segregating populations. Considering the parents as being differentiated by three pairs of genes, the genotypes and scales for the two parents, the F_1, and the two backcross populations are shown in table 2. It can be seen that the environmental variability for some genotypes of the B_1

to Johannisfeuer, the F_2, and the B_1 to Danmark populations would follow the arithmetic scale and for others the logarithmic scale. The genotypes and scales for the F_2 population are not listed since they can be derived readily from those given for the two backcross populations. Under the genetic hypothesis derived from the data of table 1, it can be determined that an increase in the number of gene pairs differentiating the parents would not change the ratio of plants following the arithmetic scale to those following the logarithmic scale for the B_1 to Johannisfeuer population. For the B_1 to Danmark and the F_2 populations, however, an increase in the number of genes differentiating the parents would increase the proportion of those following the arithmetic scale. A 1:1 ratio would be approached.

Also, it can be seen from table 2 that one *A* gene to any two other genes represented by capital letters causes the environmental variability of plants possessing them to follow the logarithmic scale. When there is a deviation of this ratio in favor of *B* and *C*, however, the environmental variability of plants possessing such genotypes follows the arithmetic scale. Likewise, the environmental variability of plants of genotypes not possessing at least one *A* follows the arithmetic scale.

The data for determining the manner in which the effects of the genes accumulate are given in table 3. First the means, both arithmetic and logarithmic, for the cross involving Danmark × Johannisfeuer show that phenotypic dominance is partial. This could be due to the fact that, the greater the number of genes acting in the same direction, the smaller the effect of any given gene (RASMUSSON's interaction hypothesis [16]). The effects of the genes could be forming a geometric progression, the sum of which is equal to the difference between the means of the two parents. Then, S_n equals $a + ar + ar^2 + ar^3 + \ldots + ar^{n-1}$, in which S_n is the sum of the progression, a is the first term, and r is the common ratio. S_n equals $a(1 - r^n)/(1 - r)$, and a equals $S_n(1 - r)/(1 - r^n)$.

The method of determining the contribution of each additional gene to weight per locule will be illustrated for a hybrid in which the parents are differentiated by three pairs of genes. In such a case n is six. An underestimate of r can be obtained from the formula $r = (\overline{F}_1 - \overline{P}_1)/\overline{P}_2 - \overline{P}_1)$. By substitution and solving the equation, $r = 0.630885$. By solving the equation $a = S_n(1 - r)/(1 - r^n)$, the first term of the progression is found to be 2.091901 gm. per locule. The value of r must be such that the first three terms of the progression will equal $\overline{F}_1 - \overline{P}_1$, which in this case is 3.35 gm. per locule (table 3). The contribution of each additional gene to weight per locule in

TABLE 2

Johannisfeuer	
aabbcc	Arithmetic
B_1 to Johannisfeuer	
aabbcc	Arithmetic
aabbCc	Arithmetic
aaBbcc	Arithmetic
aaBbCc	Arithmetic
Aabbcc	Logarithmic
AabbCc	Logarithmic
AaBbcc	Logarithmic
AaBbCc	Logarithmic
F_1	
AaBbCc	Logarithmic
Danmark	
AABBCC	Logarithmic
B_1 to Danmark	
AaBbCc	Logarithmic
AaBbCC	Arithmetic
AaBBCc	Arithmetic
AaBBCC	Arithmetic
AABbCc	Logarithmic
AABbCC	Logarithmic
AABBCc	Logarithmic
AABBCC	Logarithmic

grams for different trial values of r is shown in table 4.

In the first trial the sum of the first three terms was too high, equaling 4.24 gm. per locule instead of 3.35 gm. The second trial was too low, equaling 3.20 gm., but the third trial was sufficiently close, as the sum of the first three terms was 3.36 gm. In all cases the total effect of the genes adds to $\overline{P}_1 - \overline{P}_2$, which is 5.31 gm. per locule for the Danmark × Johannisfeuer hybrid. For those genotypes in which the environmental variability followed the logarithmic scale the effects of each additional gene were determined in a similar manner, and the data for the value of r, giving the sum of the first three terms equal to $\overline{F}_1 - \overline{P}_1$, are listed in the fifth column of table 4.

EMERSON and EAST (3) used the expansion of the binomial as a tool in the study of continuous variation. In the present study the expansion of the binomial is used to determine the number of different phenotypic classes, the proportion of each phenotype expected in the population, the scale appropriate for measuring the environmental variability of each theoretical genotype on the basis of the hypothesis advanced, and the theoretical means of the genotypes. Since the data indicate that the *Aa* gene pair conditions a scale of measurement different from that for the other pairs, it is desirable to illustrate the application of the use of the binomial in making the above determinations. Again, three pairs of genes will be used to illustrate the procedure.

In table 5 the numbers of individuals

TABLE 3

MEANS AND STANDARD ERRORS OF ORIGINAL INDIVIDUAL-PLANT DATA EXPRESSED IN GRAMS, MEANS AND STANDARD ERRORS OF LOGARITHMS OF ORIGINAL INDIVIDUAL-PLANT DATA, AND NUMBER OF PLANTS PER POPULATION

POPULATION	MEAN		NUMBER OF PLANTS
	Arithmetic (Gm.)	Logarithmic (Logarithms of grams)	
Johannisfeuer	4.61±0.446	0.612356±0.036228	452
B_1 to Johannisfeuer	6.72± .425	.777214± .021381	928
F_1	7.96± .419	.861250± .023913	469
F_2	8.35± .467	.874792± .018177	932
B_1 to Danmark	8.32± .399	.870293± .021854	921
Danmark	9.92±0.691	0.951353±0.032347	456

TABLE 4

ADDITIONAL GENES	ARITHMETIC			LOGARITHMIC
	r=0.630885	r=0.869565	r=0.833333	r=0.712708
1	2.091901	1.220086	1.330626	0.112080426
2	1.319749	1.060944	1.108855	.079880569
3	0.832610	0.922560	0.924046	.056931487
4	0.525281	0.802226	0.770038	.040575502
5	0.331392	0.697588	0.641698	.028918468
6	0.209070	0.606598	0.534748	0.020610411

in the population for the *aa* and *AA* genotypes are the coefficients of the expansion of the binomial, and the numbers of dominant genes are the exponents of *h*. For example, row heading "x^4" under *aa* has no dominant genes, and row heading "x^2h^2" under *aa* has two dominant genes. The numbers of individuals in the population for the *Aa* genotypes are two times the coefficients of the expansion of the binomial. As previously stated, the environmental variability of all genotypes possessing *aa* would follow the arithmetic scale, and that of all genotypes possessing *AA* would follow the logarithmic scale. For those genotypes of table 5 listed under *Aa*, when the exponent of *h* is 2 or less (i.e., 2 or less of *B* or *C* genes), the environmental variability will follow the logarithmic scale, and when the exponent of *h* is greater than 2, the environmental variability will follow the arithmetic scale. The mean values are obtained by adding to the mean of Johannisfeuer the respective effect of gene substitution given in table 4. For example, the first dominant gene substitution adds 1.33 gm. per locule, and this added to the mean of Johannisfeuer (4.61) gives 5.94, the mean value of table 5 listed under *aa* and row heading "x^3h." In calculating the theoretical frequency distributions, those classes having the same means, as regards both scale and magnitude, are combined in the tables which follow. The method of determining the number of individuals in the population, scale of measurement (arithmetic or logarithmic), and theoretical means as determined by the expansion of the binomial (table 5) has been illustrated for the F_2 generation. It is equally applicable to the backcross generations, however, and for any number of gene pairs differentiating the parents.

TABLE 5

NUMBER OF INDIVIDUALS IN POPULATION, SCALE OF MEASUREMENT (ARITHMETIC OR LOGARITHMIC), AND THEORETICAL MEANS AS DETERMINED BY EXPANSION OF THE BINOMIAL FOR F_2 GENERATION, SEGREGATION OF *Aa* GENES BEING SHOWN

Expansion of binomial	Segregation of *Aa* pair of genes								
	aa			*Aa*			*AA*		
	No.	Scale	Mean (Gm.)	No.	Scale	Mean (Log & gm.)	No.	Scale	Mean (Log)
x^4	1	Arithmetic	4.61	2	Logarithmic	0.724436	1	Logarithmic	0.804317
x^3h	4	Arithmetic	5.94	8	Logarithmic	0.804317	4	Logarithmic	.861250
x^2h^2	6	Arithmetic	7.05	12	Logarithmic	0.861250	6	Logarithmic	.901824
xh^3	4	Arithmetic	7.96	8	Arithmetic	8.74	4	Logarithmic	.930742
h^4	1	Arithmetic	8.74	2	Arithmetic	9.38	1	Logarithmic	0.951353

The partitioning method of POWERS *et al.* (15) was used in analyzing the data. In their paper two ways of determining the theoretical frequency distributions were given. In the first case, illustrated by measurements on percentage of flowers that set fruit, it is desirable to have the measurements on the scale appropriate for describing the biological processes responsible for the production of the character under study. The second case, as illustrated by number of locules, accepts the scale followed by the two back-

cross generations and therefore is not dependent upon determining the scale of measurement suitable for describing the character. In the present article the appropriate scales of measurement had been determined (**14**) and were employed in the gene analysis.

The obtained frequency distributions, the theoretical frequency distributions based on one, two, three, four, and ten pairs of genes conditioning weight per locule, degrees of freedom, chi-square values, and *P* values for the Danmark × Johannisfeuer hybrid grown in 1938 are given in table 6. The theoretical frequency distributions based on the assumption that the parents are differentiated by two pairs of genes give an acceptable fit for all three segregating generations. The data are not discriminatory in ruling out the possibility that three pairs of genes could be differentiating the parents, as the frequency distributions based on such an assumption give a fair fit with the obtained frequency distributions.

The theoretical frequency distributions of table 6 for one pair of genes were calculated on the assumption that *Aa* was the only pair of genes that had any influence on weight per locule. It was assumed, however, that both the *Aa* and the *Bb* gene pairs differentiated the scale of measurement in the manner shown in table 2. The assumption that only one pair of genes differentiated both weight per locule and the environmental scale is not in agreement with the data of table 1, because the data show that both scales of measurement must be involved in all segregating populations. Consequently, in testing whether weight per locule is differentiated by only one pair of genes, to be logical it is necessary to assume at least two pairs of genes differentiating the scale of measurement and that only one has any effect on weight per locule. It can be seen from the data of table 6 that the frequency distribution of the F_2 population is not in agreement with this hypothesis.

The data are fairly convincing that Danmark and Johannisfeuer, as regards weight per locule, are differentiated by comparatively few pairs of genes, probably two or three. These same pairs of genes are conditioning the scales of measurement followed by the environmental variability.

Danmark × Red Currant

In a previous publication (**14**) it was reported that, after combining the first two classes, the environmental variability followed the logarithmic scale for all six populations (both parents, F_1, F_2, and the two backcross populations). The obtained frequency distributions, the theoretical frequency distributions based on five, ten, fifteen, twenty, and forty pairs of genes conditioning weight per locule, the degrees of freedom, chi-square values, and *P* values for the hybrid Danmark × Red Currant grown in 1938 are given in table 7.

A study of table 7 shows that data for all three segregating populations lead one to reject the hypothesis that weight per locule is conditioned by as few as five pairs of genes. The F_2 data indicate that the parents are differentiated by forty or more pairs of genes. The fit between the obtained and theoretical frequency distributions for the grand total, based on forty pairs of genes, is good. The chi square for forty pairs of genes totaled 20.789 for the three segregating populations, and the *P* value for 15 degrees of freedom lies between 0.20 and 0.10. Since *L. pimpinellifolium* and *L. esculentum* have only twelve pairs of chromosomes, it is apparent that some of the pairs of

TABLE 6

OBTAINED FREQUENCY DISTRIBUTIONS, THEORETICAL FREQUENCY DISTRIBUTIONS BASED ON 1, 2, 3, 4, AND 10 PAIRS OF GENES CONDITIONING WEIGHT PER LOCULE, DEGREES OF FREEDOM, CHI-SQUARE VALUES, AND *P* VALUES FOR HYBRID DANMARK × JOHANNISFEUER GROWN IN 1938

POPULATION	NO. OF PAIRS OF GENES	UPPER LIMIT OF CLASS IN GRAMS AND LOGARITHMS, RESPECTIVELY																							DEGREES OF FREEDOM	CHI SQUARE	*P* LIES BETWEEN
		2.5 0.397940	3.5 0.544068	4.5 0.653212	5.5 0.740363	6.5 0.812913	7.5 0.875061	8.5 0.929419	9.5 0.977724	10.5 1.021189	11.5 1.060698	12.5 1.096910	13.5 1.130334	14.5 1.161368	15.5 1.190332	16.5 1.217484	17.5 1.243038	18.5 1.267172	19.5 1.290035	20.5 1.311754	21.5 1.332438	22.5 1.352182	23.5 1.371068	24.5 1.389166			
B_1 to Johannisfeuer Obtained.....	.....	52	80	106	134	131	113	84	71	56	40	21	6	10	7	5		7	3		2						
Theoretical*.....	1	76	84	125	145	135	104	77	50	37	26	19	13	9	7	6	4	3	2	2	1	1		2	13	19.7806	0.20 & 0.1
Theoretical*.....	2	62	77	121	142	138	113	87	61	44	28	18	12	7	6	4	2	2	1	1		1		1	13	8.1774	.90 & .80
Theoretical*.....	3	55	74	120	141	138	116	92	66	45	30	19	11	7	5	3	2	1	1	1		1			12	8.2390	.80 & .70
Theoretical*.....	4	54	73	118	140	139	119	94	67	46	30	18	11	6	4	3	2	1	1	1			1		12	8.9590	.80 & .70
Theoretical*.....	10	50	70	117	140	139	121	96	70	48	31	19	11	6	4	2	1	1	1	1					12	11.6870	0.50 & 0.30
F_2 Obtained.....	.....	19	35	76	103	112	110	103	82	65	40	55	42	23	21	8	14	10	5	2	3	1		3			
Theoretical*.....	1	46	58	94	117	121	106	89	71	56	44	33	25	18	14	10	6	6	4	3	2	2	1	6	16	37.6510	0.01 & ....
Theoretical*.....	2	33	47	84	109	117	110	97	80	64	50	38	29	20	15	10	7	6	4	3	2	2	1	4	16	16.2944	.50 & 0.30
Theoretical*.....	3	31	44	80	105	116	110	101	84	68	53	40	30	20	15	10	6	5	4	3	2	1	1	3	15	15.7284	.50 & .30
Theoretical*.....	4	30	43	78	104	115	112	101	85	70	54	41	31	21	15	10	6	5	3	2	2	1	1	2	15	14.9972	.50 & .30
Theoretical*.....	10	28	40	74	101	113	111	102	89	75	58	44	33	21	15	9	6	4	3	2	1	1		2	15	16.1404	0.50 & 0.30
B_1 to Danmark Obtained.....	.....	19	46	66	78	107	118	111	76	75	55	53	39	27	20	7	11	5	3	1	1	2		1			
Theoretical*.....	1	15	28	56	84	99	105	99	90	75	64	48	39	28	21	17	11	9	7	6	4	4	2	10	17	30.0814	0.05 & 0.02
Theoretical*.....	2	15	27	56	83	97	101	96	87	75	64	52	42	31	24	18	13	9	7	6	4	4	2	8	17	30.8378	.05 & .02
Theoretical*.....	3	21	27	53	77	92	97	96	88	79	67	54	44	33	25	18	13	9	7	5	4	3	2	7	17	31.3564	.02 & 0.01
Theoretical*.....	4	23	27	51	75	88	97	95	89	79	69	57	45	34	26	18	13	9	6	5	4	3	2	6	17	33.4586	.01 & ....
Theoretical*.....	10	26	27	50	71	86	94	95	89	81	71	58	47	36	26	19	13	9	6	5	3	2	2	5	17	35.5198	0.01 & ....

* In calculating the chi-square values, when necessary, the classes were grouped as indicated so that combined the obtained and theoretical averaged at least ten individuals per class.

genes are carried on the same chromosome. Likewise, many more than forty pairs of genes may be differentiating weight per locule in the segregating populations of the hybrid involving Danmark and Red Currant, because in such a study it is not possible to distinguish between blocks of fairly closely linked genes and individual pairs of genes. In either event, however, the data are rather conclusive in showing that a polygenic system is involved in differentiating weight per locule in the Danmark × Red Currant cross.

Johannisfeuer × Red Currant

The analysis of the data to determine the scale of measurement appropriate for describing the environmental variability for the populations of the hybrid, Johannisfeuer × Red Currant, is given in a previous publication (**14**). The calculation of the theoretical frequency distributions was based on the scales of measurement found to be appropriate. The obtained frequency distributions, the theoretical frequency distributions for five, ten, and twenty pairs of genes, degrees of freedom, chi-square values, and *P* values

TABLE 7

OBTAINED FREQUENCY DISTRIBUTION, THEORETICAL FREQUENCY DISTRIBUTIONS FOR 5, 10, 15, 20, AND 40 PAIRS OF GENES, DEGREES OF FREEDOM, CHI-SQUARE VALUES, AND *P* VALUES FOR HYBRID DANMARK × RED CURRANT GROWN IN 1938

Population	No. of pairs of genes	Upper limit of class in logarithms of grams													De-grees of freedom	Chi square	*P* lies between
		0.307940	0.544068	0.653212	0.740363	0.812913	0.875061	0.929419	0.977724	1.021189	1.060698	1.096910	1.130334	1.161368			
B_1 to Red Currant																	
Obtained		922	8	2													
Theoretical*	5	906	21	4	1										1	7.251	0.01 & ....
Theoretical*	10	919	11	2											1	0.396	.70 & 0.50
Theoretical*	15	923	8	1											1	0.000	.... & .99
Theoretical*	20	925	6	1											1	0.534	.50 & .30
Theoretical*	40	926	6												1	1.009	0.50 & 0.30
F_2																	
Obtained		697	147	51	25	4	4	2			1	1					
Theoretical*	5	591	161	82	44	23	12	7	5	2	2	1	1	1	5	48.553	0.01 & ....
Theoretical*	10	612	177	78	35	15	7	4	2	1	1				4	21.661	.01 & ....
Theoretical*	15	623	184	74	30	12	5	2	1	1					4	15.426	.01 & ....
Theoretical*	20	629	187	72	27	10	4	2	1						4	12.802	.02 & 0.01
Theoretical*	40	639	192	67	22	8	3	1							4	10.852	0.05 & 0.02
B_1 to Danmark							1										
Obtained		119	187	165	169	120	66	37	27	15	11	5	2	5			
Theoretical*	5	142	176	166	131	96	67	46	31	21	16	10	7	19	11	25.362	0.01 & ....
Theoretical*	10	116	185	185	146	103	69	43	29	18	12	7	5	10	10	8.194	.70 & 0.50
Theoretical*	15	106	186	193	152	106	68	43	28	17	10	6	4	9	10	7.134	.80 & .70
Theoretical*	20	101	187	197	156	107	69	43	26	16	9	5	4	8	10	7.196	.80 & .70
Theoretical*	40	94	189	205	161	108	69	42	25	14	8	5	3	5	10	8.928	0.70 & 0.50
Grand total																	
Obtained		1738	342	218	194	124	70	39	27	15	12	6	2	5			
Theoretical*	40	1659	387	272	183	116	72	43	25	14	8	5	3	5	10	12.288	0.30 & 0.20
		Value obtained by totaling degrees of freedom and chi squares for 40 gene pairs.													15	20.789	0.20 & 0.10

* In calculating the chi-square values, when necessary, the classes were grouped as indicated so that combined the obtained and theoretical averaged at least ten individuals per class.

are given in table 8. The data for B_1 to Red Currant and for F_2 populations show that the two parents are differentiated by more than five pairs of genes, and probably by more than twenty pairs of genes. This latter statement is evident from the fact that the obtained frequency distribution for the F_2 generation has so few plants in the fourth, fifth, and sixth classes as compared with the theoretical frequency distribution based on twenty pairs of genes. The obtained frequency distribution for the F_2 population has more plants in the first class and fewer in the second class than the theoretical frequency distribution based on twenty pairs of genes. Such being the case, it is evident that the obtained frequency distribution will not give a good fit with a theoretical frequency distribution based on more than twenty pairs of genes.

The situation described immediately above is that expected if both major genes and polygenes are differentiating weight per locule in the segregating populations of the Johannisfeuer × Red Current hybrid. The major genes reducing locule size must have entered the cross from the Johannisfeuer parent and must be few in number to cause so many individuals to fall in the lower classes of the F_2 population. The number of such major gene pairs differentiating the parents probably is not greater than two or three. This same shifting of the plants to the lower classes is noticeable also in the

TABLE 8

OBTAINED FREQUENCY DISTRIBUTIONS, THEORETICAL FREQUENCY DISTRIBUTIONS FOR 5, 10, AND 20 PAIRS OF GENES, DEGREES OF FREEDOM, CHI-SQUARE VALUES, AND *P* VALUES FOR HYBRID JOHANNISFEUER × RED CURRANT GROWN IN 1939

POPULATION	NO. OF PAIRS OF GENES	UPPER LIMIT OF CLASS IN GRAMS AND LOGARITHMS, RESPECTIVELY										DEGREES OF FREEDOM	CHI SQUARE	*P* LIES BETWEEN
		2.5 0.397940	3.5 0.544068	4.5 0.653212	5.5 0.740363	6.5 0.812913	7.5 0.875061	8.5 0.929419	9.5 0.977724	10.5 1.021189				
B_1 to Red Currant														
Obtained		233												
Theoretical*	5	226	7									1	7.1068	0.01 & ....
Theoretical*	10	232	1									1	1.0022	0.50 & 0.30
Theoretical*	20	233												
F_2														
Obtained		164	44	13	2	2								
Theoretical*	5	107	66	30	13	6	2	1				3	35.5716	0.01 & ....
Theoretical*	10	106	73	31	10	3	1	1				2	32.9964	.01 & ....
Theoretical*	20	101	88	27	7	2						2	36.4554	0.01 & ....
B_1 to Johannisfeuer														
Obtained		13	49	79	41	23	15	7	3					
Theoretical*	5	10	50	64	48	28	15	7	4	4		6	4.0158	0.70 & 0.50
Theoretical*	10	8	46	68	52	30	15	6	3	2		6	4.3816	.70 & .50
Theoretical*	20	6	44	70	55	31	14	6	2	2		5	5.0906	0.50 & 0.30

* In calculating the chi-square values, when necessary, the classes were grouped as indicated so that combined the obtained and theoretical averaged at least ten individuals per class.

B_1 to Johannisfeuer population. It is not sufficiently pronounced, however, for chi square calculated from the detailed frequency distribution to be discriminatory. If the first three classes are grouped to make one class, however, and the last five grouped to make another class, the chi-square value (3.906 with 1 degree of freedom) has a P value slightly less than 0.05. Hence, the obtained frequency distribution of the B_1 to Johannisfeuer population supports the conclusion drawn from the F_2 data: namely, that both major genes and polygenes differentiate the Johannisfeuer and Red Currant parents and that the pairs of major genes are few in number and the pairs of polygenes large in number.

Discussion

Of the earlier work showing that a number of genes differentiate a character, that of NILSSON-EHLE (**10, 11, 12**) is classical. HAYES (**4**), EAST (**1, 2**), EMERSON and EAST (**3**), and others showed that, at least in some cases, the quantitative characters are differentiated by a large number of genes (multiple factor hypothesis). That genes having major effects are also responsible for the differentiation of quantitative characters has been shown by the researches of HAYES and HARLAN (**5**), WARREN (**18**), LINDSTROM (**6, 7**), and POWERS (**13**).

Of the more recent work showing that polygenes (multiple factors) are involved in differentiating the quantitative characters, that of MATHER (**8, 9**) and that of STRAUS and GOWEN (**17**) are of particular interest. MATHER showed that the number of abdominal chaetae in *Drosophila melanogaster* was differentiated by a large number of genes, any one of which by itself must be having an extremely small effect. STRAUS and GOWEN, studying heterosis in egg production for a cross between two inbred strains of *D. melanogaster*, showed an increase in egg production of almost 100% above the average of the parents. Genetic analysis demonstrated that significant amounts of heterosis were contributed by each chromosome pair. The total heterotic increase equaled the sum of the individual effects; that is, no interaction or combination effects between genes carried on different chromosomes could be detected. Moreover, the heterosis due to the individual chromosomes proved to be proportional to their active length as measured by band number in the salivary chromosomes and by crossover units. These results show that a large number of genes must be conditioning heterosis in this *Drosophila* cross and that they must be scattered fairly uniformly along the active length of the chromosomes to give the observed effects.

The data for the Danmark × Johannisfeuer hybrid are most logically interpreted on the basis that the parents are differentiated by two or three major gene pairs; the data for the Danmark × Red Current hybrid, on the basis that the parents are differentiated by polygenes (ten or more pairs of genes). Probably all the twelve pairs of chromosomes of this latter cross carry genes differentiating weight per locule. Accordingly, the findings for weight per locule of tomato fruits in the Danmark × Red Currant hybrid have a great deal of similarity to the findings for egg production in *D. melanogaster* (**17**). It should be pointed out, however, that in the present study there was no direct proof that every chromosome carried genes conditioning weight per locule. As would be expected from a study of the Danmark × Johannisfeuer and the Danmark × Red Currant hybrids, the parents of the Johannisfeuer × Red Currant hybrid were found to be differentiated by a large number of genes, and both types of genes, those individ-

ually having major effects and those individually having minor effects, were found to be segregating in the hybrid populations. The genes having major effects were found to be few, whereas those having minor effects were found to be numerous. The minor genes, collectively, had a greater effect on weight per locule than did the major genes, collectively. It is interesting that both of these gene systems are conditioning weight per locule in the same cross and can be detected.

Furthermore, the data are of interest because of the bearing they have on whether it is possible in quantitative inheritance studies to distinguish between fairly closely linked blocks of genes and individual genes. In this study it was not possible to do so. As far as recombination is concerned, the results are similar whether fairly closely linked blocks of genes or individual genes are involved. In so far as theoretical genetics is concerned, it would be desirable to distinguish between the two systems.

For those hybrids in which the environmental variability is considerably greater than the variability attributable to differences between genotypes, it seems that the partitioning method is not so discriminative as are the methods available for making gene analyses of qualitative characters. This fact is brought out by the data of table 7, which show that the backcross generation data fit several genetic hypotheses almost equally well.

Summary

1. The parents involved in the gene analysis of weight per locule in tomato hybrids were Danmark (*Lycopersicon esculentum* Mill.), Red Currant (*L. pimpinellifolium* Mill.), and Johannisfeuer (*L. esculentum* Mill.). These were crossed in all possible combinations, and the P_1, B_1 to P_1, F_1, F_2, B_1 to P_2, and P_2 populations for each cross were included in the study.

2. A method of determining the contribution of each additional gene to weight per locule is illustrated for a hybrid in which the parents are differentiated by three pairs of genes. It can be applied to those cases in which the parents are differentiated by any number of pairs of genes.

3. The use of the expansion of the binomial for the following purposes is illustrated: To determine the number of different phenotypic classes, the proportion of each phenotype expected in the population, the scale appropriate for measuring the environmental variability of each theoretical genotype on the basis of the hypothesis advanced, and the theoretical means of the genotype.

4. The data for the Danmark × Johannisfeuer hybrid are most logically interpreted on the basis that the parents are differentiated by two or three major gene pairs; and the data for the Danmark × Red Currant hybrid, on the basis that the parents are differentiated by polygenes (ten or more pairs of genes).

5. As would be expected from a study of the Danmark × Johannisfeuer and the Danmark × Red Currant hybrids, the parents of the Johannisfeuer × Red Currant hybrid were found to be differentiated by a large number of minor gene pairs, and both types of genes, those individually having major effects and those individually having minor effects, were found to be segregating in the hybrid populations. Only a few (probably two or three) major pairs of genes were segregating in the hybrid populations of the Johannisfeuer × Red Currant cross.

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PELTASTROBUS REEDAE: A NEW SPHENOPSID CONE FROM THE PENNSYLVANIAN OF INDIANA

ROBERT W. BAXTER

Introduction

Several years ago the author and Dr. H. N. ANDREWS made an extensive collection of coal balls at a large strip mine approximately 10 miles north of Booneville, Indiana. The mine (Wasson Coal Mining Co.) is located in the upper part of Warrick County, and the coal is regarded as Petersburg or Alum Cave (Indiana no. 5 coal). This is correlative with the Harrisburg (Illinois no. 5 coal) and is approximately upper-middle Pennsylvanian in age. The material proved excellent and so far has yielded a wide variety of unusually well-preserved Pennsylvanian plant remains.

The present account (which we hope to be the first of a series on plants from this locality) is concerned with several specimens of a small *Sphenophyllum* or *Calamites* cone to which our interest was first attracted by the unusual preservation of the spores in which nuclei are to be clearly distinguished.

Mr. R. M. KOSANKE of the Illinois Geological Survey, to whom we showed photomicrographs of the spores and nuclei, drew our attention to an article by Dr. FREDDA REED (11) in which sporangia with similar spores were described. Her description and illustrations were clearly of a similar fructification

from Illinois coal balls. Owing to the fragmentary nature of her material, however, Dr. REED limited herself to a brief description and illustration, stating that, "with the information at hand it does not seem wise to establish a new genus for the reception of these clusters of sporangia; hence they are simply recorded as new fructification fragments possessing Calamitean characters."

Although our material also leaves a good deal to be desired in its completeness, we do feel that it presents enough additional evidence to warrant the creation of a new cone genus, which we have named *Peltastrobus reedae* in recognition of Dr. REED's original account.

Description

Peltastrobus gen. nov.—A small cone consisting of whorls of six to nine peltate sporangiophores each bearing thirty to forty sporangia in two to three concentric cycles on the adaxial margin of the peltate disk. The outer whorl of sporangia pedicellate, the inner whorls short stalked or sessile; lacking associated sterile bracts.

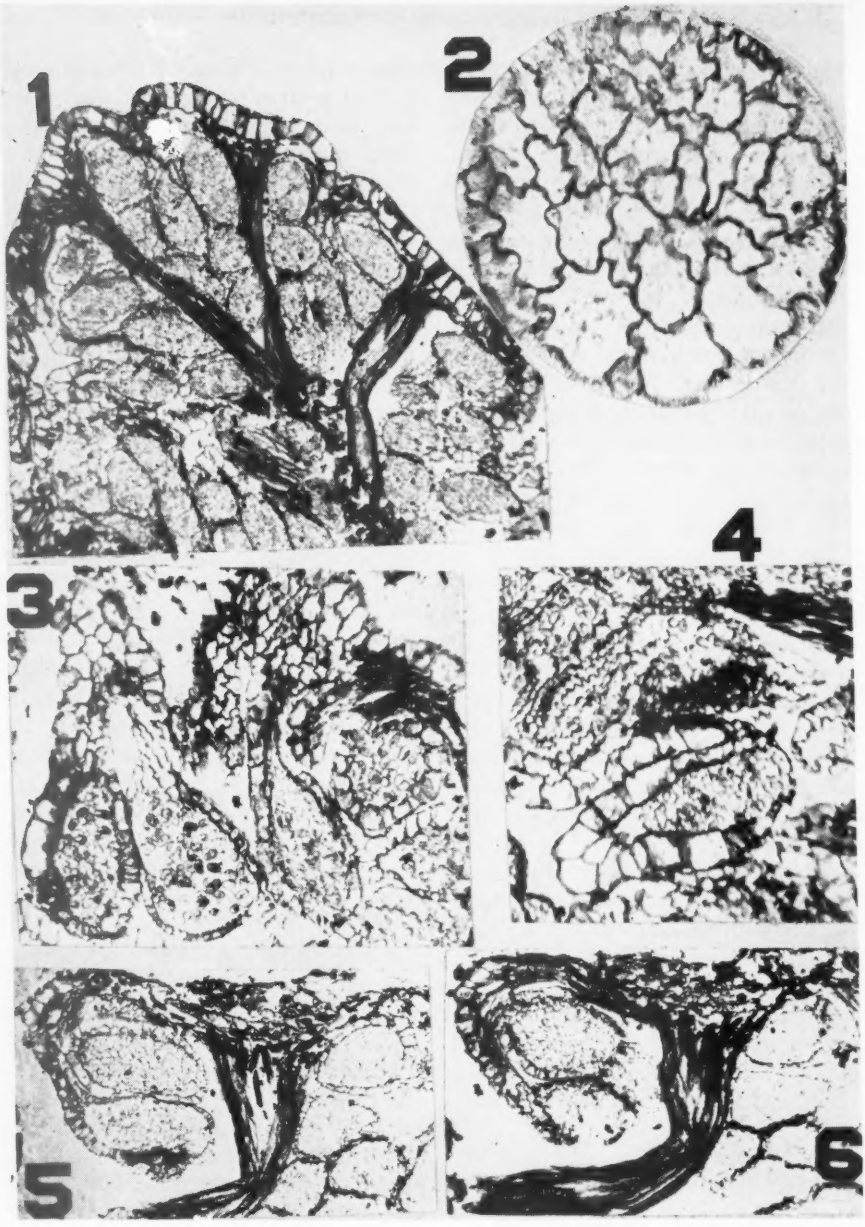
Peltastrobus reedae spec. nov.—Cone very small, not exceeding 4 mm. in diameter with sporangiophores 1.6 mm. in length. Numerous opaque secretory cells in sporangiophore stalk. Sporangial walls one cell thick, cells sinuous in face view, spores monolete, elongate-elliptical with no evident sculpturing of the walls.

Horizon: Upper-middle Pennsylvanian.

Type: Slides 101–102, paleobotanical collection, Kansas University, Lawrence, Kansas.

As in REED's material, ours also does not permit the observation of any complete cones. Several peels, however, have shown strong evidence for the arrangement of the sporangiophores into a cone-like structure. Figure 1 illustrates a portion of a cross section of a cone which had three sporangiophores in median longitudinal section and fragments of at least four to five other peltate disks with their associated sporangia. From the spatial geometry of this specimen it seems likely that there were nine sporangiophores in the whorl. The axis of the cone was evidently very small, since, where the position is not disrupted, the stalks of the sporangiophores all appear to meet at a small common central point. This possibly accounts for the fact that the strobili are always found in such fragmentary condition. The individual sporangiophores, when seen in median longitudinal section, exhibit a stalk averaging 1.6 mm. in length and 0.22 mm. in width at the base. They widen gradually toward the distal end, where the sporangiophore gives rise to a peltate expansion which averages 1.5 mm. in diameter when measured at right angles to the stalk from one incurved sporangia-bearing lobe to another directly opposite. The longitudinal view of the sporangiophore stalk shows four to six centrally placed tracheal elements with annular thickenings inclosed by a cortical region five to six cells thick. The cells of this latter tissue are quite long and resemble the vascular tissues in both diameter and length. They show, however, no evidence of pitting or secondary wall sculpturing.

Immediately surrounding the conductive tissue is a loose sheath of elongated secretory or resin cells which are always completely opaque and dark brown or black in color. At the distal end, where the sporangiophore becomes peltate, these conductive, secretory, and cortical cells are seen to radiate out at right angles to the sporangiophore stalk forming the inner (adaxial) surface of the expanded apex. The outer (abaxial) surface



FIGS. 1–6.—*Peltastrobus reedae* Baxter. Fig. 1, cross section of portion of cone showing three sporangiophores with attached and associated sporangia. From slide 101. ×31. Fig. 2, face view of sporangial epidermal cells showing sinuous shape. From slide 103. ×71. Fig. 3, tangential cut through margin of peltate disk showing at left two sporangia of outer cycle. From slide 104. ×58. Fig. 4, sporangium in lower center shows decrease in size of wall cells from point of attachment to distal end. From slide 105. ×58. Figs. 5, 6, exact median-longitudinal view of single sporangiophore showing attachment of inner and outer whorls of sporangia. From slide 102. ×42.

is covered by a single layer of very large cells, 150 $\mu \times 75$ μ in side view, arranged in a palisade fashion with their longer axis at right angles to the plane of the inner tissues. In face view these cells present a very sinuous appearance (fig. 2).

The sporangia are borne in two to three concentric whorls on the inner surface of the peltate expansion. The outermost sporangia are formed by a lobing of the disk margin in which the layer of palisade cells appears to become double and to form the sporangial walls (fig. 3). As a result the cells in the wall of the upper half of these outer sporangia are often equal in size to those of the abaxial surface of the sporangiophore but gradually diminish in size toward the unattached end (fig. 4).

The inner whorl of sporangia arises where the large palisade cells first appear as a double layer, and their wall cells tend to be smaller and more uniform. Whereas the sporangia in the outer whorl often appear stalked as a result of the considerable incurving and elongation of the marginal lobes, the inner whorl appears nearly sessile (figs. 5, 6).

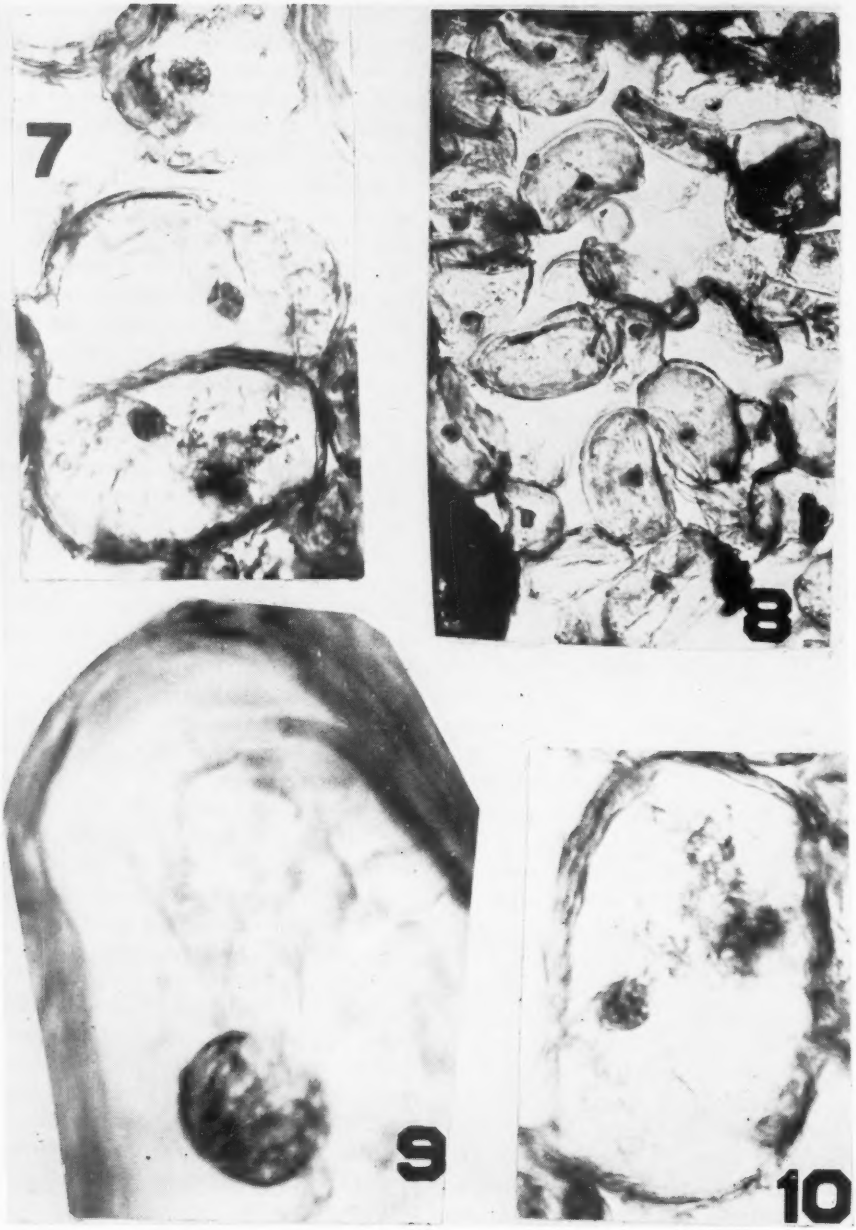
The sporangial walls in all cases are just one cell thick. The sporangia are oval to slightly elongated, averaging 0.6 mm. $\times$ 0.4 mm. $\times$ 0.3 mm. The spores are numerous, monolete, elongate-elliptical, and show no evidence of wall sculpturing. They average 37 $\mu \times$ 22 μ in our material, which possibly represents an immature stage, since REED has indicated that in the Illinois specimen they averaged 50 $\mu \times$ 25 μ. The spores fall in the *Laevigatosporites* type.

As mentioned previously our attention was first attracted to the specimen in question by the constant occurrence of well-preserved nuclei in the spores. Figures 7–10 illustrate with unretouched photomicrographs the remarkable detail and preservation of the nuclear structures. Descriptions of fossilized nuclei and chromosomes have appeared previously in the literature (**4, 5, 14, 15**), but there has always seemed to be a considerable amount of skepticism in the minds of many paleobotanists that the proof was conclusive. Needless to say, in light of the present direct photographic evidence we do not feel that doubt about the matter can any longer be justified. The nuclei are constant, one to each spore, and of uniform size, being spherical to slightly oval in shape and averaging 3.6 μ to 5.4 μ in diameter. Many of them show well-defined chromatin granules (figs. 9, 10). A nuclear membrane (sometimes ruptured) is present. The nuclei are quite constant in their position within the spore, being held (with few exceptions) by the peripheral cytoplasm at the center of the spore on the inner surface (with reference to the original tetrad) so that they lie opposite the middle point of the monolete suture. Dr. EDGAR ANDERSON has been kind enough to look over several of the original peel slides and concurs with the author that the structures undoubtedly represent nuclei.

Thus the degree of fixation and preservation of carboniferous plants in coal balls can be definitely recognized as potentially equal to that obtainable by modern microtechnique methods with living plants. It is hoped that this fact, once known and accepted, will encourage many more workers in the field to look for histological and cytological details in addition to investigating the gross morphology and anatomy of the fossilized plant remains.

Discussion

The morphology and phylogeny of the sphenopsid cones, both living and fossil, have been discussed at length by numer-



FIGS. 7–10.—*Peltastrobus reedae* Baxter. Fig. 7, three spores with nuclei. ×1167. Fig. 8, general view of spores showing constancy of occurrence of nuclei. ×542. Fig. 9, portion of single spore enlarged to show details of nucleus. ×3500. Fig. 10, single spore showing nucleus with chromatin granules. ×1750. From slide 106.

ous investigators (**3, 6, 9, 13, 16**). In large part the problem has been one of interpreting the origin and relationships of the fertile sporangiophores and the sterile bracts and of deciding whether the bractless condition is primitive or reduced. The bracts and sporangiophores have variously been regarded (**3**) "as lobes of a dorsiventrally divided sporophyll, or the bracts . . . as sterilized sporangiophores, or the sporangiophores . . . as fertile leaves."

Considering the two major orders in which sterile bracts are usually present (the Sphenophyllales and the Calamitales), a fairly general distinction has been made in that the former are held to bear the sporangia or sporangiophores on the bract, while in the latter the sporangiophores are attached directly to the cone axis either above or in the axil of the bract. Also the calamitalian sporangiophores were thought to be constantly peltate, although recent investigation (**9**) has shown them to be cruciate. The calamitalian fructifications usually bear four sporangia on a sporangiophore, while the sphenophyllalian counterpart may have from one to two sporangia, although in two cases (*Bowmanites majus* and *Cheirostrobus*) four sporangia per sporangiophore have been described.

To illustrate further the complexity of the problem, WALTON (**16**) has attributed all bract-sporangiophore associations in the Sphenopsida to a division of "pinnate sporophylls" in which some pinnae became sterile (bracts) while others remained fertile (sporangiophores). On the basis of this theory he made the following statement:

> When the vascular systems supplying the sporophylls of *Sphenophyllum fertile* and *Cheirostrobus* are compared a striking similarity is apparent. They may both be interpreted as derivatives of pinnate sporophylls; in *Cheirostrobus* three and in *S. fertile* two of the basal pinnae being sterile and assuming a dorsal position and the distal pinnae fertile and assuming a ventral position.

On the other hand, BROWNE (**3**) and SCOTT (**12**) stressed the similarities of *Cheirostrobus* to *Calamostachys* on the basis of characters of the sporangiophore with its four sporangia. Regarding this, SCOTT has stated: "In its peltate sporangiophores, and in the insertion and details of structure of its sporangia, *Cheirostrobus* agrees exactly with the *Calamarieae*."

Obviously much of this apparent disparity in opinion arises from the nature of *Cheirostrobus* itself; it seems, however, also to indicate that the relationships among the various sphenopsid fructifications may be generally closer than has been previously assumed. Thus, although REED in her original account of our new genus suggested a calamitean affinity, we are inclined, on the basis of the following evidence, to consider it closer to the Sphenophyllales.

First, the nature of the sporangia. SCOTT (**12**) wrote of the sporangia of *Sphenophyllum* (*Bowmanites*) *dawsoni:*

> In the upper part of the pedicel, and especially where it bends over towards the axis, the epidermal cells are much enlarged, and these large cells of the pedicel are continuous with those that form the wall of the sporangium itself. Towards the free end of the sporangium the cells of the wall become much smaller.

It can be seen by referring to our description of the *Peltastrobus* sporangia that the characters are almost identical.

Second, the nature of the sporangiophore and sporangial epidermal cells. WALTON (**16**) stated regarding *Bowmanites römeri:*

> The epidermal cells of the upper part of the pedicel had sinuous walls. . . . The epidermis of *S. cuneifolium* and *S. majus* is constructed of

the same type of cell, a fact which gives supporting evidence in favor of attributing *Bowmanites römeri* to the Sphenophylleae.

As noted in the description and illustrated in figure 2, *Peltastrobus* has precisely this type of sinuous epidermal cell.

Third, association of parts and the similarity of the secretory cells in *Peltastrobus* with those in a *Sphenophyllum* leaf. Association of various plant organs in the coal ball is, of course, a rather unreliable feature on which to base relationships. REED's original tentative assignment of *Peltastrobus* to the Calamitales was, however, in part made on the basis of close association with *Calamites* stems and leaves. In contrast, in our material, *Calamites* stems and leaves are completely lacking, while in almost every case the cone fragments are in close association with small *Sphenophyllum* stems and leaves. Accordingly, although the association itself may be of doubtful value, in this case it did serve to emphasize certain striking similarities between the secretory cells surrounding the vein in a *Sphenophyllum* leaf and the same cells surrounding the vascular tissue in the *Peltastrobus* sporangiophore. The opaque "resin cells" are of identical size and shape in both organs.

Fourth, the similarity of the concentric whorls of stalked sporangia in *Peltastrobus* and *Bowmanites*. HOSKINS and CROSS (7) indicated that at least nine of their eighteen listed species had sporangia in two or more concentric whorls and also that one of the "outstanding characters" delimiting *Sphenophyllum* fructifications from the allied calamitean genera is "the pedicellate or stalked nature of the sporangia."

Thus we are confronted in *Peltastrobus* with a considerable number of *Sphenophyllum* attributes in spite of the absence of the subtending sterile bracts which typify the genus *Bowmanites*. This lack of intervening bracts or sporophylls is a condition which so far has only been found in the calamitean genus *Protocalamostachys* (17) and possibly *Asterocalamites*. Both of these genera are of much greater age (Lower Carboniferous) than *Peltastrobus* and have been cited as evidence for the primitiveness of the bractless state. However, the fact that this primitive character was never completely lost in the evolution of the sphenopsid cone is borne out by the strobilus of *Equisetum* which is held (1) to be quite possibly a "primitive organ derived from the fertile branch tip of the Psilophytales."

Regardless of what stand one takes on the nature of the fertile and sterile parts of the many fossil sphenopsid cones, there is certainly strong evidence that in some of the most primitive mid-Devonian genera attributable to the Sphenopsida (*Hyenia* and *Calamophyton*) the sporangia were borne on dichotomously forked determinate branches or telomes with no trace of sterile bracts or sphorophylls. In addition, the recent work of LACEY (9) seems to show that the supposed peltate structures in *Calamostachys* are really cruciate and of a form which can be clearly compared to a double dichotomy of the *Calamophyton* sporangiophore. Accordingly the condition in *Peltastrobus* may possibly represent a holdover of primitive characters in the sphenophyllalian line in which a profuse ramification of a *Hyenia*-like fertile telome gave rise to a peltate disk with a series of marginal sporangia. While both REED'S specimens and our present material are from the upper-middle Pennsylvanian, recent collections from the Fleming and Mineral coal of Kansas have produced unmistakable *Peltastrobus* sporangiophores. This hori-

zon is lower-middle Pennsylvanian and indicates a considerable geological range for the genus.

The close relationship and undoubted common ancestry of the Calamitales and Sphenophyllales have previously been pointed out by various authors (**2, 8**), as has also the primitiveness of the cone without associated sterile bracts. It therefore seems not unreasonable to postulate a close and common origin of fructification types from such simple forms as *Hyenia* and *Calamophyton*. To attribute structures arising from "pinnate sporophylls" to a group which is characterized only by simple dichotomy and whorled arrangement of lateral organs seems to be straining unnecessarily for a connection of the Sphenopsida to the ferns—a relationship for which there seems little fossil evidence. If, on the other hand, we accept the evidence for origin from the above mid-Devonian genera (or similar forms), we could then hold either that the bracts are the result of the complete sterilization of alternating sporangiophore whorls (fertile telomes) with eventual vertical reduction (*Paleostachya*), or that they result from the sterilization of the lower branches of a profusely dichotomized fertile ramulus (*Bowmanites fertilis et al.*). The sporangiophores also are basically telomic, consisting of whorls of short determinate fertile branches in which the subsequent developmental lines have depended on whether the sporangia were borne one or more to a dichotomy (*Calamophyton* vs. *Hyenia*).

The stelar anatomy of forms such as *Cheirostrobus* and *Sphenostrobus thompsonii* (**10**) certainly present real difficulties in assigning them to either the Calamitales or the Sphenophyllales. Some years ago, however, the late Professor SAHNI showed the author sketches of tetrarch and pentarch steles which he felt showed in the main many *Sphenophyllum* characters. It therefore seems doubtful as to whether a fructification should be ruled out completely from the latter group on the basis of unconforming stelar structure.

Although in cases where the cone axis is known the calamitalian fructifications appear to have stelar structure similar to that of the stem, the sphenophyllalian fructifications show much more variability. *Bowmanites dawsonii*, fairly definitely of *Sphenophyllum* affinity, has a hexarch stele which, although reported for vegetative stems of *Sphenophyllum*, is certainly not common.

Summary

1. A new genus of sphenopsid fructifications is described from upper-middle Pennsylvanian coal balls.

2. Nuclei in the spores are described and illustrated.

3. The affinities of the new genus are discussed and the conclusion reached that the evidence points toward a closer relationship to the Sphenophyllales than to the Calamitales.

4. The morphological implications of the sterile bracts and sporangiophores are discussed with respect to their phylogenetic development.

DEPARTMENT OF BOTANY
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A COMPARISON OF SEASON OF CAMBIAL GROWTH IN DIFFERENT GEOGRAPHIC RACES OF PINUS PONDEROSA

R. F. DAUBENMIRE

Introduction

Ecotypic specialization in different parts of the ranges of widely distributed species is a phenomenon that has commanded the attention of botanists for several decades. Experience has usually shown that when plants are moved from their native habitat into a different environment they become subject to injuries from factors associated with the new environment, although occasionally they may prove more successful. The slow genetic fitting of plant populations to the peculiarities of the area which they have occupied for a long time is especially important in the artificial establishment of forests, for usually there are available for use in plantations seed lots representing the same species but from populations attuned to widely different environments. The long life-cycle of the tree makes it possible to invest much time and money in reforestation before genetic weakness becomes apparent; moreover, the unsuccessful planting may be expensive to remove, and by cross pollination it may seriously contaminate near-by native trees. In recognition of this problem the United States Forest Service established an experimental plantation of *Pinus ponderosa* Laws. in northern Idaho, with the purpose of determining the relative value of seeds from different parts of its area for use in reforestation in northern Idaho.

Between 1911 and 1917 seeds or seedlings from twenty-two localities were planted in contiguous plots at the Priest River Experimental Forest in Bonner County, Idaho. The habitat is an upper river terrace mantled with sandy loam. Climate and soil in this region permit *P. ponderosa* to grow well under open conditions, but the environment is too cool for

satisfactory natural germination or satisfactory growth after the forest approaches maturity. This choice of location for the experiment has considerable bearing upon the practical application of the findings[1] but is of little consequence from the purely scientific standpoint; for, although the climate here may generally be unsuited for the commercial planting of this species, it is at least conducive to the growth of the trees in young plantations and therefore may be considered adequate for a comparative test of racial differences under uniform environment.

Twenty-two to 26 years after the seeds germinated the results were described by WEIDMAN (6). His report described the origin of the populations, climates of those regions, arrangement of the plantation, native vegetation and soils, and the following attributes of the populations: number of needles in the fascicle, length and persistence of needles, anatomy of needles, general appearance of foliage, and heights and diameters at ages of 22 or 23 years. In summarizing all the observations, WEIDMAN tentatively grouped the populations into four geographic races.

Some of the populations planted at the Priest River Experimental Forest have suffered very high mortality, and one (Shasta) was completely exterminated by a sudden drop of temperature from 7° to −25° C. within 20 hours in December, 1924. Appraisal of injury that may be attributed specifically to this sudden drop in temperature showed that the Siskiyou, Santa Fe, and Coconino races also suffered heavily, although devastation was not complete as with the Shasta race (4).

[1] Another study of ten populations of *P. ponderosa* from different regions, in which each population was distributed among six widely different habitats, showed that relative rates of height growth cannot be predicted for one habitat on the basis of comparisons made on another (5).

The hypothesis has been advanced that trees suffer from autumnal frost as a result of continuing some phase of growth activity too late in the season (3, 7), and WEIDMAN suggested this as a possible explanation for heavy mortality in some races: "Presumably the long period of growth activity characteristic of . . . Siskiyou progeny, has been one of the causes of the frost damage suffered by the latter in northern Idaho" (6:879). One of the objects of the present study was to determine if the races experiencing high mortality terminate radial growth of their stems relatively late in the season.

Studies by the writer of a number of species of trees growing some 177 km. (110 miles) south of the Priest River Experimental Forest indicated that day-length exerts a marked control over the date of commencement of cambial growth in some species, although this was not true of the specimens of *P. ponderosa* of unknown and possibly different geographic origin which were included in that study (2). It was evident that the collection of races of known origins at the Priest River Experimental Forest representing nearly 14° of latitude could provide conclusive information on this problem, and, consequently, this question represents a second object of the present study.

Since WEIDMAN's report did not include records of the season of cambial activity, permission was requested to study the trees from this standpoint. The writer is grateful to the United States Forest Service for making the experimental plots available and for assistance in reading dendrometers which the writer installed. Further support was given the project by funds provided for biological and medical research by the State of Washington Initiative Measure N. 171.

Figure 1 shows the total geographic area of *P. ponderosa* (incl. var. *scopulorum* Engelm. which the southernmost stations, at least, represent) and the points of origin of the populations selected for the present study. The populations are referred to by name of the National Forests in which they were collected.

Methods

Dial-gauge dendrometers of a type (1) which the writer has compared with similar instruments and found eminently more successful were used in this investigation. Instruments were installed in the autumn of 1947 and records maintained throughout 1948. During the period

FIG. 1.—Origins of populations considered in this study, as related to total range of *Pinus ponderosa*, including var. *scopulorum*.

when growth was expected to begin, readings were made at intervals of usually less than 1 week, but at other seasons longer periods elapsed between observations, depending upon the state of cambial activity.

For most trees of temperate latitudes, day-to-day diameter increase is a regular phenomenon only during the main period of growth. During the dormant season trunks commonly exhibit periods of shrinkage which are associated with series of dry days in the latter half of the summer, or subfreezing weather in winter. They may also swell intermittently. It is consequently very difficult to establish a precise date for the beginning and end of growth, but the date for completion of 5% and 95% of the season's increment may be approximated by interpolation, with very little probability of error owing to the smoothness of the curve once the cambium becomes active. Radii as measured on December 30, 1947, and on November 12, 1948, seemed least affected by frost shrinkage, and the differences between these readings were used in calculating growth on a percentage basis.

Results and discussion

A striking feature of the results was the variation exhibited by different trees from the same point of origin. In other studies by the writer three vigorous trees selected to represent a population have always showed reasonably close similarity in behavior, but at the Experimental Forest there was so much divergence among individuals which averages would obscure that, in presenting the results, the data for each tree are kept separate. It is obvious that statistical significance cannot be claimed for any of the results, but since they, for the most part, reveal little differences among the populations, the main question which prompted the study can be answered with reasonable assurance of accuracy. The conclusions which may be drawn from the data represented by figure 2 are as follows.

Nearly all populations began growth after mid-May and completed growth in late August. Median dates for the 5%, 50%, and 95% levels were May 23, June 21, and August 22. The Coconino population was an exception in that its cambial growth began about 2 weeks later[2] than the average for all groups. The midpoint in its growth was proportionately late as well. The Harney population, represented by only two trees, also began growth distinctly later than average for all groups. Here, however, the rate of growth was exceptionally rapid once the cambium became active, so that its midpoint was not delayed and, in fact, was reached earlier than in certain of the other groups.

The Helena, Bitterroot 2200 m. (7200 ft.), and possibly Ashley populations all terminated their growth a little in advance of the others, although they had not begun to grow earlier in spring than most of the others. Except for one tree, the growing season of the Coconino population was shorter than average as a result of postponement of inception in spring, whereas the Helena, Ashley, and Bitterroot 2200 m. races exhibited slightly shortened growing seasons owing to earlier cessation of growth in late summer.

The Bitterroot populations representing different altitudes (1200 [4000 ft.] and 2200 m.) differed only in that the race from the higher altitude ceased growing earlier. In view of this evidence of racial differentiation in *P. ponderosa* according

[2] Lateness also characterizes the beginning of apical growth in this population (6).

to altitude, all results from the comparative plantation may be strongly conditioned by the elevation from which the populations originated. However, the physical data of altitude that are available (6) cannot be interpreted closely because the character of biologic environment in mountains depends upon the direction of slope exposure as much as upon elevation above the seas. The environment of the plantation at the Priest River Experimental Forest is close to the upper zonal (not altitudinal) limits of *P. ponderosa* in the Southern Selkirks, whereas the original habitats of the populations may have been from the upper, middle, or lower parts of the total elevational ranges in the other mountain systems. Therefore, the change in zonal status in some cases may have reduced and in others augmented the temperature difference involved in latitudinal displacement.

WEIDMAN (6) suspected that the heavy mortality suffered by the Siskiyou population subsequent to planting might have resulted from too late an extension of the growing season in the autumn, for this population originated from a very mild climate. The present study, how-

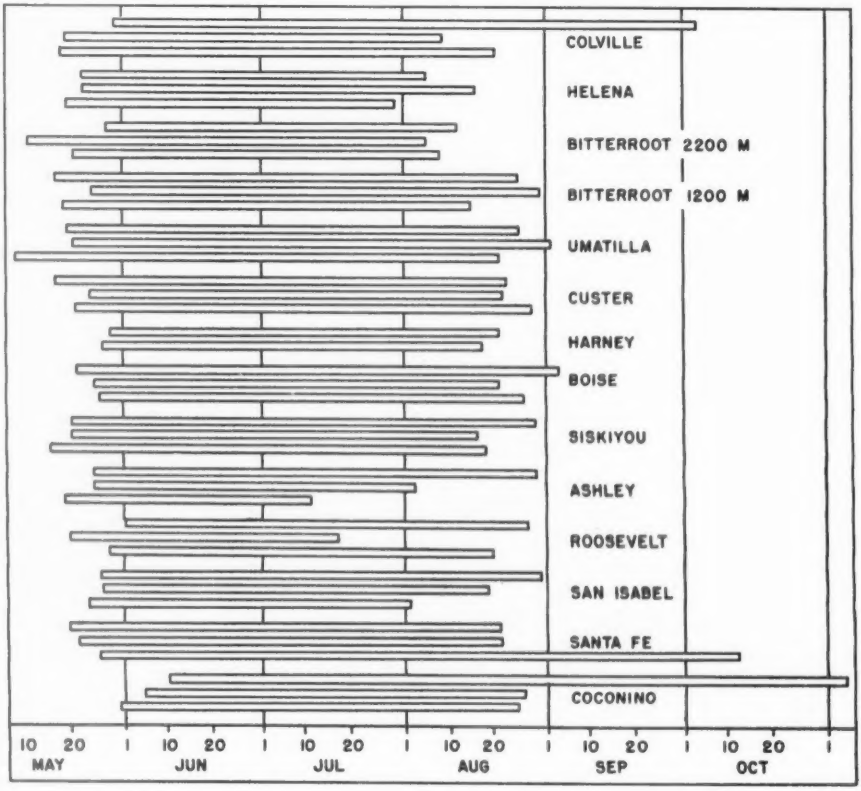

FIG. 2.—Principal period of cambial activity of each tree studied, based on period starting when 5% of total annual increment was recorded and ending when increment was 95% completed.

ever, does not substantiate this hypothesis. Among the Siskiyou, Bitterroot high altitude, Santa Fe, and Coconino populations, all of which have suffered high mortality, it might be suspected that climatic selection has eliminated biotypes that continued growth relatively late. But the fact that three of the forty-one trees studied continued cambial growth a full month later than all others without exhibiting ill effects therefrom indicates that the others do not exploit the potential growing season to the extent of its possibilities. If selection had operated upon this character, one would expect the survivors of the decimated populations to include a high percentage of individuals that continue growth rather late. But this is definitely not true. If the deceased trees were ones that continued growth late, they must have differed to a very great extent from the survivors. The conclusion therefore seems warranted that the termination of cambial activity is strongly determined by autogenous forces and occurs well in advance of the onset of critically low temperatures. Climatic selection must have operated on physiologic characters other than lateness of cambial activity.

Although there is considerable difference among individuals within one population, there is a striking similarity in periods of radial growth among populations originating in widely different climatic types and latitudes. This contrasts sharply with the relatively uniform appearance of trees within each population but distinct difference in appearance among the populations (6).

Deviations of particular populations from the growth pattern of the species as a whole exhibit no geographic trends. Daylength either does not determine the beginning of cambial activity or on this habitat other conditions masked the influence of daylength. Since the results of earlier studies (2) of a population of unknown origin growing in a grassland climate also furnish negative evidence of photoperiodic control over inception of cambial activity, the former of the two possibilities mentioned above appears to be correct.

Summary

1. Radial growth was studied in forty-one trees including *Pinus ponderosa* Laws. and its var. *scopulorum* Engelm., during the calendar year 1948. These trees represented populations obtained from known sources over a wide area in the western United States and had been growing on the same habitat at Priest River Experimental Forest in northern Idaho since 1911–1917.

2. Median dates for completion of 5%, 50%, and 95% of total radial growth were May 23, June 21, and August 22. Although there was considerable variation in growing seasons within populations, there was but little difference among the populations. Susceptibility to frost damage does not appear to be correlated with radial growth that continues late in the season. In these fourteen populations representing nearly 14° of latitude, daylength appears to exert no influence upon the growing season. Duration of cambial activity is not related to grouping of the populations into races on the basis of morphology and anatomy.

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EFFECT OF 2,4-D ON CARBOHYDRATE AND NUTRIENT-ELEMENT CONTENT AND ON RAPIDITY OF KILL OF SOYBEAN PLANTS GROWING AT DIFFERENT NITROGEN LEVELS[1]

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Introduction

The physiological response of plants to the selective herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is for the most part an unexplained phenomenon. Some species are readily affected, whereas others are more or less resistant. Most of the grasses are particularly resistant to injury from the usual applications of 2,4-D. Occasional informal reports have indicated that rapidly growing plants are more easily destroyed by 2,4-D than are those growing slowly. Some investigators (**4**, **5**, **8**) have suggested that the herbicidal properties of 2,4-D are related to the carbohydrate storage and nitrogen metabolism of plants.

In an experiment to determine the relative effectiveness of 2,4-D on soybean plants growing at different nitrogen levels, a pilot experiment was conducted by the senior author in 1947. Duplicate pots of Chief soybeans were grown in the greenhouse in sand culture at three nitrogen levels—high (336 p.p.m.), medium (56 p.p.m.), and low (14 p.p.m.)—and then a drip aqueous solution of 20 p.p.m. 2,4-D (the sodium salt of 2,4-D was used) was added to the cultures. Plants growing on high nitrogen were affected much more rapidly than were those growing at low and medium levels. Plants growing on low nitrogen were least affected, and the plants on medium nitrogen were between the low- and high-nitrogen plants in response to 2,4-D.

The results of this test were of sufficient interest to warrant further study of the effect of applications of 2,4-D on plants growing at different nitrogen levels. Therefore, an attempt has been made to relate growth status with the speed and intensity of plant response to 2,4-D applications and to determine certain associated physiological changes.

Material and methods

Three plants of the Lincoln variety of soybeans were grown in sand culture in the greenhouse in each of twelve glazed 2-gallon pots from February 27 to April 23, 1948. Until the plants reached the six-leaf stage, a uniform nitrogen level was maintained by flushing the sand

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, departments of Farm Crops and Plant Physiology.

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daily with a complete nutrient solution containing 56 p.p.m. of nitrogen. Different nitrogen levels were then introduced (table 1), referred to as low (14 p.p.m.), medium (56 p.p.m.), and high (336 p.p.m.). Sulfate and chloride were necessarily varied in order to provide the wide variations in nitrogen and still maintain nearly constant osmotic pressure in the nutrient solutions. The constant-renewal method as described by SHIVE and ROBBINS (7) was used, 2 quarts of solution being applied per pot each 24-hour period.

Three weeks after the different nitrogen levels were first established, the plants receiving low-nitrogen solution showed some chlorosis owing to nitrogen starvation. At this time the application of 2,4-D was begun. The sodium salt of 2,4-D was dissolved in distilled water in a quantity to produce a solution containing 20 p.p.m. of acid equivalent of 2,4-D. This solution was applied at the same rate and by the same method as the concomitantly supplied nutrient solutions. The same amount of distilled water was supplied to cultures which did not receive 2,4-D.

To decrease the possibility of 2,4-D coming in contact with the aerial parts of the soybeans, the surface of the sand was covered with nonabsorbent cotton, and the outlet from the syphon tubes supplying the 2,4-D was kept under this cotton. All the solution passing through the sand was collected by means of tubing leading from the opening at the base of the pot to a large stoppered bottle. Tomatoes, which are highly sensitive to 2,4-D, were grown on an adjacent bench without visible symptoms of 2,4-D injury.

Flower buds were removed as soon as they were formed on untreated plants. No flower buds were formed on the plants treated with 2,4-D. No nodules were present on any plants grown in this experiment.

Daily observations were made to determine the effect of the 2,4-D on the plants. Epinasty, chlorosis, splitting of stems, and drying of leaves were used as criteria of response.

The application of 2,4-D was continued for 14 days, at the end of which time the foliage of the plants grown on the high-nitrogen level was dry and apparently dead. The plants were then harvested, and the stems and leaves (including petioles) were separated and cut into 1-cm. lengths. This tissue was dried in a forced-draft oven at 70° C. for 48 hours. After drying the samples were ground in a Wiley mill to pass an 80-mesh screen, and replicates of each treatment were combined. The composite samples were analyzed for starch, total sugars, reducing sugars, hemicellulose, potassium, calcium, magnesium, phosphorus, sulfur, total nitrogen, and chlorine to determine the differences in concentration of these constituents which resulted from the treatments.

TABLE 1

CONCENTRATION (P.P.M.) OF ELEMENTS IN NUTRIENT SOLUTIONS USED IN GROWING SOYBEANS*

Nutrient	Low N	Medium N	High N
Nitrate—N	14	56	336
Sulfate—S	224	160	64
Cl	228	245	0

* Concentrations of all other elements were constant and supplied at the rate of 168 p.p.m. Mg, 195 p.p.m. K, 200 p.p.m. Ca, 31 p.p.m. P, ½ p.p.m. Fe, ½ p.p.m. Mn, ½ p.p.m. Zn, and ½ p.p.m. B.

CARBOHYDRATE DETERMINATIONS.—The soluble carbohydrates were extracted by reflux with 80% alcohol, centrifuging, and decanting off the supernatant liquid according to the methods of SMITH

et al. (8). The alcoholic extract was analyzed for sugars and the residue for starch and hemicellulose.

Reducing sugars were then determined by the WILDMAN and HANSEN semimicro method (11), modified to suit the needs of the present study by reducing the volume of aliquot from 20 ml. to 5 ml. and the normality of the potassium dichromate from 0.1 to 0.05. The results following this modification were in good agreement with those obtained with the original method.

Total sugars were obtained by enzymatic inversion at 38° C. for 3 hours with Wallerstein's red-label invertase (1:134). Reducing sugars were then determined as invert sugar.

Enzymatic hydrolysis of the alcohol-insoluble residue with fresh saliva according to the method of SMITH *et al.* (8) was used in the determination of starch. The reducing sugars were then determined, and 90% of the value was reported as starch.

The residue from the starch hydrolysis was treated with 2.5% hydrochloric acid for 2 hours at 95° C., and the reducing sugars were determined and reported as hemicellulose.

CATION AND ANION DETERMINATIONS.—Analyses for potassium, calcium, magnesium, phosphorus, and sulfur were made according to the procedures outlined by TOTH *et al.* (9). Total nitrogen was determined by the Kjeldahl procedure modified to include nitrate (1:27). Chlorine was determined by titration with silver nitrate with a chromate indicator after dry-ashing in the presence of calcium carbonate (1:128). A positive test for nitrates by the diphenylamine test was found only in the stems of the high-nitrogen control plants.

The cations and anions were calculated as milliequivalents per 100 gm. of dry tissue as described by WALLACE and BEAR (10); i.e., K^+, Ca^{++}, $H_2PO_4^-$, SO_4^{--}, NO_3^-, and Cl^-, according to the valences of the ions encountered in the nutrient solution. Total cations, anions, and cation-anion ratios were calculated for leaves and stems combined. The cation-anion ratio in this paper is used to mean the ratio in the plant of the concentration of the nutrient cation elements to the concentrations of the nutrient anion elements, each element being expressed in terms of the ion in which it is present in the nutrient solution.

Results

GROSS RESPONSE

When treatment with 2,4-D was begun, the leaves of plants receiving low-nitrogen solution were mildly chlorotic and had developed a brown spotting of the lower leaves. This spotting spread, and some of the lower leaves dried and were abscised. This condition was aggravated by treatment with 2,4-D; many of the lower leaves of the low-nitrogen, treated plants fell off before the plants were harvested. These leaves were not included in the chemical analysis. Plants receiving medium-nitrogen solution showed normal growth, whereas the high-nitrogen plants were darker green at the time the 2,4-D was applied.

Seven days after the 2,4-D treatment was begun, epinasty of the petioles appeared in the treated plants receiving medium-nitrogen solution. Two days later severe epinasty of the petioles of the treated plants receiving high-nitrogen solution and slight epinasty of those on low-nitrogen were apparent. After this first reaction the high-nitrogen plants were rapidly affected, and on the twelfth day some chlorosis of their stems and leaves and splitting of the lower portions

of their stems occurred. The high-nitrogen plants were nearly dead 14 days after 2,4-D treatment began. At this same time the plants on medium-nitrogen showed some chlorosis of leaves and stems but no splitting of the stems. Plants growing in low-nitrogen solution were only mildly chlorotic on the fourteenth day. The rapidity and extent of injury are shown graphically in figure 1. Extent of injury was given arbitrary ratings from 1 to 10, 1 indicating the first appearance of reaction to 2,4-D, and 10 indicating complete dryness of the foliage.

Appearance of the plants on the fourteenth day after 2,4-D application was begun is shown in figure 2. Replicates of a given treatment showed similar degrees of injury. The roots (fig. 3) showed the same relative injury as did the aerial parts. Roots of treated plants receiving high-nitrogen solution were badly decomposed on the fourteenth day, those of treated, medium-nitrogen plants

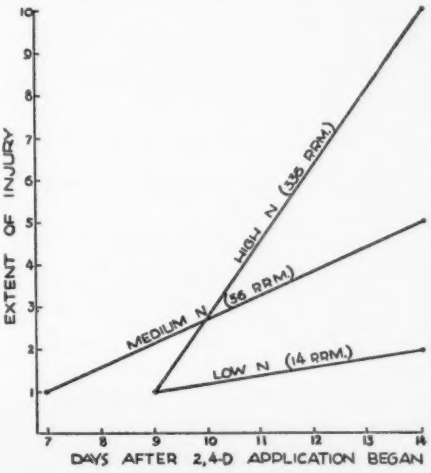

FIG. 1.—Rapidity and extent of injury to soybean foliage when 2,4-D was added to sand culture.

FIGS. 2, 3.—Soybean. Fig. 2 (*above*), *left to right*, high-, medium-, and low-nitrogen plants 14 days after their treatment with 2,4-D was begun. Fig. 3 (*below*), roots of control and 2,4-D–treated plants; *left to right*, respectively, low-nitrogen control and treated; medium-nitrogen control and treated; and high-nitrogen control and treated.

showed some decomposition, and roots of the low-nitrogen plants were nearly normal.

The proportion of leaves to leaves and stems on a dry-weight basis (leaf percentage, table 3) was reduced considerably by the 2,4-D treatment.

Carbohydrate content

Results of the carbohydrate analyses are given in table 2.

Hemicellulose.—The percentage of hemicellulose was higher in the leaves and stems of the 2,4-D–treated plants than in the control plants at each of the nitrogen levels. This higher percentage of hemicellulose represented the most consistent difference between treated and untreated plants. The greatest difference appeared in the leaves of the medium-nitrogen plants, where the hemicellulose content was 2.34% in the control and 3.36% in the 2,4-D–treated plants. Whether these higher percentages in the treated plants represented an actual increase in hemicellulose or were a reflection of their lower total dry weight is not known.

TABLE 2

NITROGEN AND CARBOHYDRATES IN 9-WEEK-OLD SOYBEAN PLANTS EXPRESSED AS PERCENTAGE OF DRY WEIGHT

Treatment		N	Hemicellulose	Starch	Total sugars	Reducing sugars	Total available carbohydrates*
N level	2,4-D						
		Leaves plus stems					
Low	Control	2.39	1.84	2.31	0.69	0.53	3.26
Low	2,4-D	1.34	1.98	0.58	0.76	0.76	1.40
Medium	Control	2.49	2.43	3.63	1.62	0.90	5.65
Medium	2,4-D	1.89	3.08	2.85	1.41	1.35	4.58
High	Control	3.47	1.66	1.44	1.31	0.95	2.91
High	2,4-D	2.84	2.71	0.83	1.98	1.07	2.90
		Leaves					
Low	Control	3.55	1.76	2.30	0.58	0.58	2.87
Low	2,4-D	2.90	2.10	0.52	0.72	0.72	1.30
Medium	Control	3.22	2.34	4.61	1.74	0.70	6.86
Medium	2,4-D	1.92	3.36	3.14	0.80	0.80	4.29
High	Control	4.68	1.41	1.70	1.40	0.76	3.29
High	2,4-D	2.52	1.89	0.81	3.30	1.46	4.20
		Stems					
Low	Control	1.27	1.92	2.32	0.80	0.48	3.38
Low	2,4-D	0.90	1.95	0.60	0.78	0.78	1.44
Medium	Control	1.53	2.56	2.33	1.46	1.16	4.05
Medium	2,4-D	1.88	2.93	2.69	1.74	1.66	4.73
High	Control	2.11	1.95	1.22	1.20	1.16	2.56
High	2,4-D	3.12	3.39	0.84	0.90	0.74	1.83

* Total available carbohydrates refers here to starch as glucose plus total sugars.

STARCH.—The percentage of starch was lower in 2,4-D–treated than in untreated plants. This lower value does not seem to be correlated with the severity of 2,4-D injury, since the greatest percentage difference was in the plants grown in low-nitrogen solution. This decrease in percentage of starch following application of 2,4-D agrees with results reported by other workers. The starch content in the high-nitrogen plants at the time of death was 0.83%. It would seem unlikely that the death of these plants could be explained on the basis of a decrease in starch alone. Applications of 2,4-D have been reported to cause an increase in the rate of respiration (**2, 3, 4, 5**). An increased rate of respiration could explain the rapid decrease in starch in the treated plants.

TOTAL SUGARS.—The percentage of total sugars was somewhat higher in the leaves of the plants treated with 2,4-D than in the leaves of the respective controls of the low- and high-nitrogen groups. Total sugars were lower in the leaves of the 2,4-D–treated plants in the medium-nitrogen group than in the controls. These differences were not consistent and, therefore, cannot be explained on the basis of severity of injury from 2,4-D.

REDUCING SUGARS.—The percentage of reducing sugars in the stems and leaves was higher in the 2,4-D–treated plants than in the controls in all cases with the exception of the high-nitrogen stems. All the sugars in the leaves and stems of low-nitrogen, treated plants were reducing sugars. On a whole-plant (aerial part) basis the percentage of reducing sugars was consistently higher in the 2,4-D–treated than in the control plants. Here, again, the differences were not correlated with the severity of 2,4-D injury observed.

TOTAL NITROGEN CONTENT

The total nitrogen percentage of the leaves of the 2,4-D–treated plants was considerably lower than that in the leaves of the controls. In the stems the percentage nitrogen content was slightly lower in the 2,4-D–treated, low-nitrogen plants than in the low-nitrogen, control plants but higher in the 2,4-D–treated, medium- and high-nitrogen plants than in their respective controls. The greatest percentage difference was in the high-nitrogen plants. This suggests the possibility of (*a*) translocation of nitrogen from leaves to stems in the 2,4-D–treated plants, (*b*) failure of nitrogen to move to the leaves, or (*c*) a combination of these. In any case, these results indicate the importance of studying the effect of 2,4-D on nitrogen distribution in plants.

CATIONS AND ANIONS

The nutrient-element composition of the plants is given in table 3. These data are of value in learning the influences of variation of anions in the nutrient substrate as well as the influences of 2,4-D on plant composition.

The data show that the cation-anion ratios were essentially constant for leaves plus stems, as well as for leaves and stems separately, of control plants at all nitrogen levels. This is interesting in view of the different nutrient solutions, the many variations encountered in the chemical composition of the plants, and the variations in leaf percentage. The ratios for 2,4-D–treated plants were higher than those for control plants. Ratios in the leaves of treated plants were directly, and those in the stems inversely, related to nitrogen levels supplied in contrast to the constant ratios for each part of the untreated plants. This indicates a definite interaction between nitrogen level

and 2,4-D in their effect on mineral utilization and should be studied further.

Differential distribution of elements among various organs is an important reason for variation in the mineral composition of plants. In this respect the potassium contents of the leaves of 2,4-D–treated plants were less than those of control plants, the means being 34 and 62 m.e. per 100 gm. dry tissue. A similar decrease in potassium and no change in phosphorus or magnesium in rape treated with 2-methyl-4-chlorophenoxyacetic acid has been reported by RHODES and TEMPLEMAN (6). When leaves were combined with stems, however, these differ-

TABLE 3

NUTRIENT-ELEMENT CONTENT OF SOYBEAN PLANTS GROWN IN SAND CULTURE UNDER VARYING LEVELS OF NITROGEN AND 2,4-D IN M.E. PER 100 GM. DRY MATTER*

TREATMENT		LEAF† PERCENTAGE	K	Ca	Mg	CATION SUM	N	P	S	Cl	ANION SUM	CATION‡/ ANION
N level	2,4-D											
		Leaves plus stems										
Low	Control	48.3	82	63	75	220	171	66	25	31	293	.75
Low	2,4-D	23.8	82	70	65	217	96	44	33	43	215	1.01
Medium	Control	56.9	66	64	66	196	178	41	14	23	256	.77
Medium	2,4-D	35.2	58	57	75	190	135	32	24	28	219	.87
High	Control	53.4	73	69	82	224	248	30	11	4	293	.76
High	2,4-D	45.2	66	74	99	239	203	30	17	10	260	.92
Mean	Control	52.9	73	65	74	212	199	46	17	19	281	.75
Mean	2,4-D	34.7	69	67	80	216	145	35	25	26	231	.93
		Leaves										
Low	Control	.....	73	70	79	222	254	73	28	25	380	.58
Low	2,4-D	.....	37	103	68	208	207	54	33	41	335	.62
Medium	Control	.....	53	77	65	195	230	55	17	23	325	.60
Medum	2,4-D	.....	27	90	69	186	137	32	25	36	230	.81
High	Control	.....	60	86	91	237	334	30	14	1	379	.63
High	2,4-D	.....	38	119	111	268	180	28	18	11	237	1.13
Mean	Control	.....	62	78	78	218	273	53	20	16	362	.60
Mean	2,4-D	.....	34	104	83	221	175	38	25	29	267	.83
		Stems										
Low	Control	.....	90	54	72	216	91	60	23	36	210	1.03
Low	2,4-D	.....	95	61	65	221	64	41	33	44	182	1.21
Medium	Control	.....	83	48	68	199	109	22	11	18	160	1.24
Medium	2,4-D	.....	76	39	78	193	134	32	23	23	212	.91
High	Control	.....	87	51	71	209	151	30	8	7	196	1.07
High	2,4-D	.....	90	36	86	212	223	32	16	9	280	.76
Mean	Control	.....	87	51	70	208	117	37	14	20	188	1.11
Mean	2,4-D	.....	87	45	76	208	140	35	24	25	224	.93

* Valences used for m.e. calculation correspond to the ion present in the solution, i.e., K^+, Ca^{++}, Mg^{++}, NO_3^-, $H_2PO_4^-$, and Cl^-. To convert to percentage dry weight multiply K by 0.039, Ca by 0.02, Mg by 0.012, N by 0.014, P by 0.031, S by 0.016, and Cl by 0.0355.

† Leaf percentage refers to the proportion of leaves to leaves plus stems on a dry-weight basis.

‡ The cation-anion ratio refers to the ratio in the plant of the concentration of the nutrient cation elements to the concentration of the nutrient anion elements, each element being expressed in terms of the ion in which it is present in the nutrient solution.

ences largely disappeared. Calcium was greater in leaves of 2,4-D–treated plants than in controls, but there was little difference on the leaf-plus-stem basis. It is possible that the differences in cation-anion ratios of treated and untreated plants would likewise disappear if the roots were analyzed and the results combined with leaves and stems to represent the whole plant. Differences in the cation-anion ratio were due mainly to the nitrogen and phosphorus anions.

WALLACE and BEAR (10) have discussed cation-anion ratios in the alfalfa plant and have shown that these ratios were constant under certain conditions for whole plants but not necessarily constant for individual plant parts. These workers indicated that the equivalent cation-anion ratio could be a highly useful tool in studying certain aspects of plant nutrition. Its use in this study is an aid in noting the influences of 2,4-D on the chemical composition of soybean plants. Since roots were not analyzed, it is not possible to determine whether 2,4-D merely affected the distribution of the nutrient elements throughout the plant or influenced their relative absorption by the plant.

When analyses for leaves and stems were combined, the percentage nitrogen content of plants was directly related to concentrations of nitrogen in the nutrient solution for both treated and untreated series, although the percentage nitrogen of treated plants was much lower than that of control plants. In general, the nitrogen levels of the nutrient solution for both treated and control series were inversely related to phosphorus, sulfur, and chlorine contents of the plants. This relationship is modified somewhat by the concentration of sulfur and chlorine in the nutrient solution but is associated with the tendency of certain plants to maintain constant cation-anion ratios or, under certain conditions, cation- and anion-equivalent constancy. The chlorine and sulfur contents of treated plants were higher than those of control plants. Phosphorus paralleled nitrogen, being lower in treated leaves and in low-nitrogen stems but higher in treated, medium- and high-nitrogen stems than in their respective controls.

Discussion

A definite interaction between nitrogen level and 2,4-D in their effect on growth of soybean plants was shown by this experiment. Plants growing on high nitrogen were killed very readily by application of 2,4-D to the substrate, whereas the medium- and low-nitrogen plants were injured to a much lesser extent. This raises the question as to whether high-nitrogen plants are more sensitive to 2,4-D than are plants growing at lower nitrogen levels or whether more 2,4-D enters the high-nitrogen plants. It is conceivable that, because of the higher metabolic rate and probable higher transpiration rate in the high-nitrogen plants, more 2,4-D might enter them and thus cause quicker kill. High-nitrogen plants might also be quicker to respond because of the rapid production of new root and shoot tissues, which are readily killed by 2,4-D. Production of these new tissues was very slow in the low-nitrogen plants.

Despite the fact that the nutrient solutions supplied all plants were sufficient, with the exception of nitrogen, for normal plant nutrition, the phosphorus and sulfur contents of the high-nitrogen plants were much lower than those of the medium- and low-nitrogen plants. It is well known that in plants a very important role is played by phosphorus in energy metabolism and by sulfur in protein

metabolism. This suggests the possibility that because of the smaller percentage of either phosphorus or sulfur or both in the high-nitrogen plants, the ability of these plants to tolerate or deactivate 2,4-D was less than that of plants growing on lower nitrogen levels.

If this same interaction of nitrogen level with 2,4-D holds true when application of 2,4-D is made to foliage, degree and time of fertilization might well be very important factors in the proper use of this herbicide. Field observations in 1948 indicated that plants growing on soil which had received high-nitrogen fertilizer were, in general, more easily killed by 2,4-D than plants growing on soil which had received a low-nitrogen fertilizer. Field experiments will have to be conducted and research data collected before this is proved. Differences in levels of fertility might well be one of the important reasons for the variable plant responses obtained in field applications of 2,4-D.

The data obtained in this experiment do not point toward a definite mechanism of action of 2,4-D. It seems apparent, however, from the large percentage of total available carbohydrates remaining in the 2,4-D–treated, high-nitrogen plants, that death of the plants cannot be explained on the basis of a depletion of these food reserves. The percentage of starch was lower in 2,4-D–treated plants than in control plants, but there was little difference in percentage of total sugars. Although the percentages of total available carbohydrates were lower in 2,4-D–treated, medium- and low-nitrogen plants than in their respective controls, the percentages present in high-nitrogen, 2,4-D–treated and control plants were nearly the same. Yet the high-nitrogen control plants were growing luxuriantly after the treated plants had been killed by the 2,4-D treatment.

One of the most consistent percentage differences occurred in the hemicellulose content of the plant. In all cases hemicellulose percentage was higher in the 2,4-D–treated plants than in their respective controls. Explanation of this is not possible from the data presented here.

The much lower nitrogen percentage in the leaves of 2,4-D–treated plants, compared with controls, indicates a possible effect of 2,4-D on nitrogen metabolism or on the distribution of nitrogen. Analysis of the different nitrogen fractions should aid greatly in explanation of the physiological action of 2,4-D.

Summary

1. Soybeans were grown in sand culture with nutrient solutions containing 14, 56, or 336 p.p.m. of nitrogen. After 3 weeks of differential nitrogen applications, a solution containing 20 p.p.m. 2,4-D was applied to the sand for 14 days. The plants were then harvested and analyzed.

2. The 2,4-D–treated plants receiving high-nitrogen solutions were dead 14 days after the 2,4-D treatment was begun. At this time the medium-nitrogen plants showed severe chlorosis of leaves and stems and splitting of the stems, whereas the low-nitrogen plants showed only mild chlorosis.

3. Roots of 2,4-D–treated plants receiving high-nitrogen were badly decomposed at harvest. Roots of 2,4-D–treated, medium-nitrogen plants showed some decomposition, whereas roots of treated, low-nitrogen plants were nearly normal.

4. The hemicellulose content was higher and the starch content lower in 2,4-D–treated plants than in untreated plants.

5. Total sugars were higher in 2,4-D treated, low- and high-nitrogen plants and lower in the 2,4-D–treated, medium-nitrogen plants than in their respective controls.

6. Reducing sugars were consistently higher at each nitrogen level in 2,4-D–treated plants than in control plants.

7. At each nitrogen level both the size of the plants and the proportion of leaves to leaves-plus-stems were lower in 2,4-D–treated plants than in controls.

8. Similar cation-anion ratios were noted for untreated plants, for all nitrogen levels, for leaves, for stems, and also for leaves and stems combined. On 2,4-D–treated plants this ratio in leaves was directly related to nitrogen concentrations in the nutrient solution but was inversely related in the stems. When leaves and stems were combined, the ratios for treated plants were higher than for control plants but nearly constant among themselves. These differences in ratios were due mainly to difference in the nitrogen and phosphorus anions.

9. Potassium was considerably lower and calcium was higher in leaves of 2,4-D–treated plants than in controls, but, when results of leaves were combined with stems, these differences largely disappeared.

10. Phosphorus, sulfur, and chlorine contents in the untreated plants were inversely related to nitrogen concentration in the nutrient solution. This was also true of the treated and untreated plants if considered as separate series. At each nitrogen level chlorine and sulfur were higher in treated plants than in controls, while phosphorus was lower in treated plants than in controls of low- and medium-nitrogen level.

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ABSORPTION, TRANSLOCATION, AND PERSISTENCE OF 2,4-DICHLOROPHENOXYACETIC ACID IN SOME PLANTS[1]

A. S. DHILLON AND E. H. LUCAS

Introduction

Few attempts have been made to study the absorption and translocation of synthetic growth-regulators in plants by direct methods. ZIMMERMAN and HITCHCOCK (13), in their recent review, stated that there were only a few cases in which the applied hormone had been detected or identified by direct methods beyond the region of application. They mentioned their own work (8) of 1938 which dealt with the extraction and identification of indole compounds from plants treated with indoleacetic acid and indolebutyric acid. The more recent paper by WOOD *et al.* (12) on the application of a radioactively tagged plant growth-regulator (2-iodo-3-nitrobenzoic acid) on bean and barley leaves also indicated that the growth-regulator entered the plant in molecular form and was translocated as such.

Most of the workers who have published discussions on this subject apparently assumed that the responses observed indicated that the substances used were absorbed and translocated. A few, however, like MITCHELL and BROWN (10), who used the term "resulting stimulus" when referring to translocation phenomena, seemed to feel that they had insufficient proof for a more explicit statement. DHILLON and LUCAS (3) concluded from grafting experiments that the "formative stimulus" induced by 2,4-dichlorophenoxyacetic acid was translocated across a graft union. They refrained from defining the exact nature of the stimulus because of the lack of evidence pertaining to absorption, translocation, and persistence of the growth-regulator. BEAL (1) stated that it was not known how or in what form the substituted phenoxy compounds used in his experiments entered the plant.

In view of these observations and of hypotheses like that of GUTTENBERG (6) that synthetic growth substances are mere activators of native hormones, it is hardly advisable to draw conclusions about absorption and translocation solely on the basis of growth responses, because these may have resulted from indirect actions initiated at places other than those where final responses are exhibited. Direct approaches to a study of these phenomena by extracting from treated plants and identifying the growth-regulators applied may aid in understanding the mode of their action.

It was with these aims in mind that the present study on absorption, translocation, and persistence of 2,4-dichlorophenoxyacetic acid (applied as the sodium salt and henceforth designated as "2,4-D") was undertaken. It was felt that a study concerned with the absorption and translocation might advantageously take into consideration the entry of such materials through leaves as well as roots. In the present investigation, therefore, both leaves and roots were treated in different experiments.

[1] Journal Article no. 1131 of the Michigan Agricultural Experiment Station.

Experimentation

Absorption through roots

For studying the absorption through roots two groups of plants were used: tomatoes and kidney beans, which are susceptible to the action of 2,4-D, and sweet corn and oats, which are resistant to it. All plants were grown in the greenhouse in 4-inch unglazed clay pots containing a mixture of three parts of soil and one part of peat, from August to December, 1948. After the plants had reached the desired size, the soil was treated with different concentrations of 2,4-D after ascertaining in each case that the amount of 2,4-D added caused growth responses. Precautions were taken to avoid contamination by covering the basal parts of the stems with vaseline to prevent a possible external rise of the chemical by capillarity. The pots were covered with wrapping paper, with a small opening left for the plant, to prevent contact of the aerial parts with the contaminated soil. The following amounts and concentrations of aqueous 2,4-D solutions were used with each group of plants:

Tomatoes.—Ten ml. of a 500-p.p.m. solution of 2,4-D for individual pots containing single plants of the John Baer variety, 6 weeks old and 30–35 cm. in height.

Beans.—Twenty ml. of a 1000-p.p.m. solution of 2,4-D for each pot containing two plants of the Red Kidney bean, 12 days old, with folded trifoliolate leaves.

Corn.—Ten ml. of a 1000-p.p.m. solution of 2,4-D for each pot containing two plants of sweet corn, variety Golden Security, 8 days old, with three leaves.

Oats.—Ten ml. of a 1000-p.p.m. solution of 2,4-D for each pot containing eight plants of Kent oats, 9 days old with three leaves.

The chemical was applied to the soil surface, and the soil then saturated with water in each case. Tomatoes and beans showed nastic responses 6–12 hours after application.

In each case plants were decapitated 24 hours and 5 days, respectively, after the application. The vaseline-coated stumps were left in the pots. These stumps were 5–7 cm. long in tomatoes, with the cotyledons and the first two leaves attached; in beans, corn, and oats they were 4–5 cm. long.

The exudate from the cut surfaces of the stumps of tomato and bean plants was collected by means of dropper bulb micro-pipettes. The two saps collected were separately applied with the same pipettes to primary leaves of 9–12-day-old Red Kidney bean plants with trifoliolate leaves still in the bud stage. In addition, the color test described by Freed (5) was applied to the exudates of treated and untreated tomato plants after evaporation and further preparation according to Freed. Tomato stumps yielded a sufficient quantity of exudate to make both tests possible. The exudate from beans did not suffice for the color test, while neither corn nor oats supplied sufficient sap for either test.

Methods of extraction.—Extractions by two methods—with water and with acid and ether—were made of the tops of the plants decapitated after 24 hours or 5 days. Each sample of tomatoes comprised five plants, of beans fourteen plants, of corn fourteen plants, and of oats fifty-six plants.

In the extraction with water the plant material was crushed by means of a Waring blendor, filtered by suction through Whatman no. 2 circles, and the filtrate concentrated on a steam bath.

In the acid-ether extraction concentrated sulfuric acid was added to the crushed material to make a 10% solution

of acid. The acidified preparation was refluxed for 2 hours to hydrolyze the material. It was then filtered as with aqueous extraction, the filtrate was shaken with ethyl ether, and the ether layer separated. This procedure was repeated three times, and the ether extracts were combined.

One-third of the ether thus collected was kept for the color test. An excess of sodium bicarbonate was added to the remaining portion, and the ether was evaporated. The residue was dissolved in distilled water, and the solution adjusted to a pH value of approximately 5.5 in accordance with the pH values of aqueous extracts of treated plants. The preparations thus obtained from different plants were tested by application to the primary leaves of Red Kidney bean plants as described before.

Since the acid-ether extraction method involved the conversion of the sodium salt of 2,4-D into the acid and the subsequent reconversion to the sodium salt, a separate experiment was set up in which an aqueous solution of the commercial sodium salt was subjected to the same treatment as the plant extract. The resulting solution of sodium 2,4-dichlorophenoxyacetate was brought to approximately the theoretical concentration of the plant extracts. When tested on bean plants and by the color indicator, this solution gave positive results.

QUALITATIVE METHODS OF DETERMINING 2,4-D RECOVERED FROM PLANTS.—The ether extract obtained by the method mentioned above was evaporated in a test tube, and the color test described by FREED (5) was used. A few crystals of 1,8-dihydroxynaphthalene 3,6-disulfonic acid (chromotropic acid) and 2 ml. of concentrated sulfuric acid were introduced into the test tube containing the residue, and the mixture was gently heated in an oil bath at 140°–150° C. for 2 minutes. The development of a wine-purple color indicated the presence of 2,4-D or some other halogen derivative of phenoxyacetic acid. Since the ether extracts contained other substances whose presence interfered with the development of the characteristic color, ether extracts of untreated plants were tested as checks. The color determination was facilitated by dilution of the heated material after cooling and allowing it to stand until the suspended matter had separated.

In the other method the aqueous extract and the aqueous solution of the residue obtained from the ether extraction were applied to bean plants. Morphological changes induced in trifoliolate leaves expanding after the application were taken as an indication of the presence of 2,4-D in the extracts.

RESULTS.—Exudates from cut stumps of tomato and bean plants decapitated 24 hours after application of 2,4-D solutions to the soil in which they were growing caused epinasty and morphological changes of leaves on test bean plants when applied to their primary leaves (fig. 1). FREED's test on sap collected from the cut surfaces of such tomato stumps was also positive.

The test of extracts from the tops of these plants also showed the presence of a growth-regulator. Aqueous extracts of tomato plants made 24 hours and 5 days, respectively, after the soil treatment induced curvature and changes in the form of the trifoliolate leaves of bean plants, but such extracts of the tops of beans, corn, or oats did not induce these effects. Acid-ether extracts of all species used, however, did cause them, from plants 5 days as well as from those 24 hours after application of 2,4-D. In all aqueous and acid-ether extracts the FREED test gave positive results.

FIGS. 1, 2.—Fig. 1 (*below*), visual evidence of absorption of 2,4-D through roots. Red Kidney bean plant 2 was treated by application of six drops of exudate collected from cut stumps of decapitated tomato plants 24 hours after 10 ml. of a 500-p.p.m. aqueous solution of sodium 2,4-dichlorophenoxyacetate had been applied to soil in which tomato plant grew. Plant 1 was similarly treated with six drops of sap from stumps of untreated tomato plants. Picture taken 18 days after application. Fig. 2 (*above*), persistence of 2,4-D in plants shown by response of Red Kidney bean plants. Plants 2 and 3 were treated with ether extracts made from tissues of tomato plants to which had been applied 0.1 ml. of saturated 2,4-D solution on cut surface of stem, 26 days prior to preparation of extracts; plant 2 received extract of roots and stem base; plant 3, extract of leaves. Plant 1 treated with ether extract from untreated tomato plant. In all cases eight drops of the extracts were applied to one primary leaf. Picture taken 18 days later.

ABSORPTION THROUGH LEAVES

Attempts were made to recover 2,4-D from plants after it had been applied to the leaves. The method of application consisted in placing on the leaf blades either a few drops of a concentrated solution of 2,4-D or a mixture of the substance with oleomargarine. The latter was chosen as the carrier because of some very desirable properties: absence of stickiness, a consistency which allowed spreading at greenhouse temperature and cutting off and weighing desired quantities after refrigeration, and availability in uniform quality. It was found to be innocuous when applied to plants. The growth-regulator was admixed after gentle heating of the carrier. The treated leaves were covered with cellophane bags to avoid contamination and were separated from the plants after given periods. The tops of the plants were then extracted according to the methods previously described. None of the extracts thus obtained yielded evidence of 2,4-D when tested either on bean plants or by FREED's method.

Three possible explanations for the negative results are the following: (*a*) The chemical was not translocated from the treated leaves. (*b*) The substance was translocated and was recovered but was lost in the process of extraction. (*c*) The substance was present in too small a quantity to be recovered or to show an effect in the preparation applied. Since the untreated leaves on the treated plants showed epinasty, the first possibility can be ruled out if the nastic responses are taken to be evidence of the presence of the growth-regulator. The second possibility is unlikely, since the same procedure had yielded 2,4-D from extracts made after absorption through roots from soil. The third possibility was shown to be correct by the use of a different technique.[2]

The method of introduction of 2,4-D through leaves of tomato and corn plants is shown in figure 3. John Baer tomato plants, 3 weeks old, with three to four leaves, were used. The second basal leaves of the plants were introduced carefully, without breaking or bruising any part, into test tubes which had been inserted in the soil. The bases of the blades of the corn leaves and the petioles of the tomato leaves which remained outside the test tubes were coated with vaseline to avoid any possible effect on nonexposed parts caused by moving of the chemical on the surfaces. Some of the leaves were kept submerged in a 1000-p.p.m. solution of 2,4-D for 24 hours, and others for 48 hours. After these periods the treated corn leaves were cut at the ligules, and the tomato leaves at the bases of the petioles. Cross sections of treated leaves were examined microscopically and showed no evidence of external damage of the epidermal tissue.

For extraction each tomato plant was divided into the top, including all parts above the treated leaves, and the lower part, including the stem base with lowest leaf and cotyledons and the roots. Entire corn plants including roots were used for extraction. The two methods previously described were employed. Each sample was comprised of ten plants (or plant parts). All extracts were tested for responses of bean plants in the manner described; the acid-ether extracts were also tested by FREED's method.

Twenty-four hours after start of the

[2] After this technique was developed, the authors became acquainted with the publication "A leaf immersion technique for studying the absorption and translocation of chemicals in plants" (Contrib. Boyce Thompson Inst. 15:165. 1948) by LAWRENCE J. KING. In many respects his technique resembles the application described.

immersion treatment some of the tomato plants were decapitated above the treated leaves still attached to the stem, and the exuding sap was collected from the cut stump. This sap was applied to the primary leaves of bean plants.

RESULTS.—All acid-ether extracts prepared from tomato and corn plants after 24-hour and 48-hour leaf-immersion treatments indicated the presence of 2,4-D on the basis of the color test. Positive responses in the bean test were obtained for all acid-ether extracts except that made from the lower portion of tomato plants after 24 hours. Even this portion gave very pronounced effects, however, when it was extracted after 48 hours of leaf immersion.

Aqueous extracts produced similar effects when made from tops of tomato plants after 24-hour leaf immersion and from both tops and lower parts after 48-hour treatment. The aqueous extracts from treated corn plants showed no activity. Aqueous extracts were not tested by FREED's method.

Exudates from stumps of tomatoes decapitated after a 24-hour immersion treatment gave positive results in the bean test as well as in the color test. The treated leaves when examined microscopically and compared with normal leaves showed no signs of injury or exposure of the vascular tissue. It is probable that, owing to the high concentration of the growth-regulator and the considerable amounts taken in, leakage into the xylem and upward transport in the stem might have occurred.

PERSISTENCE OF 2,4-D IN PLANTS

After thus establishing that 2,4-D enters the plant and is translocated probably as such, it appeared desirable to ascertain whether it would remain within the tissues for an extended period. For this purpose tomato plants of the variety John Baer, 7 weeks old, were chosen. Three healthy leaves were left on each plant which was decapitated just below

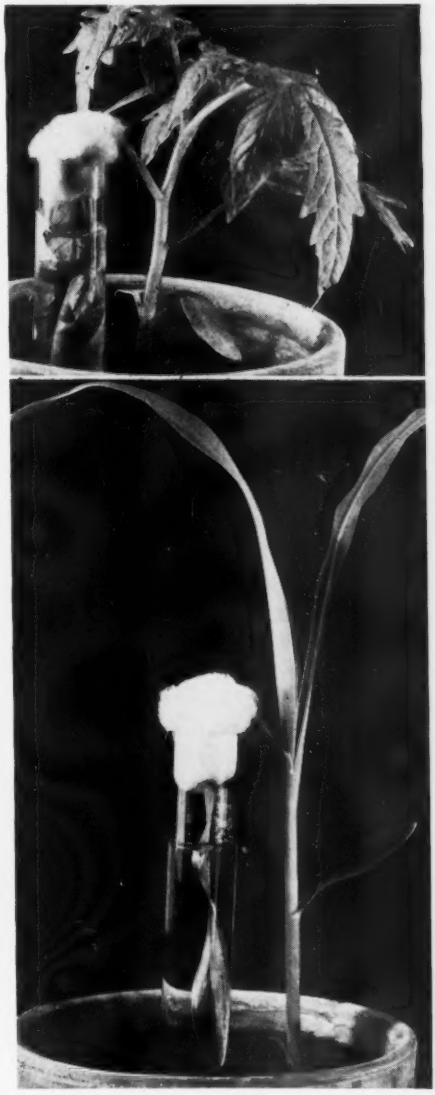

FIG. 3.—Immersion technique used for purpose of incorporating measurable quantities of 2,4-D into plants.

the fourth leaf. A hole approximately 2 cm. deep was bored in each stump downward from the cut surface. Four drops, approximately 0.1 ml., of a saturated aqueous solution of 2,4-D were placed in the holes by means of dropper bulb pipettes, taking great care that no external contamination occurred. The treated part of the stem and an additional piece, 3 cm. long, were removed 24 hours after treatment.

Samples of six plants each were taken 24 hours, 7 days, and 26 days after treatment. The plants were divided into leaves, upper and lower halves of stems, and roots each time, and extractions of these portions were made separately by the acid-ether method. The only difference between these preparations and those mentioned earlier was that the hydrogen-ion concentration was adjusted to pH 7. The extracts were tested on Red Kidney bean plants and also by the FREED method.

RESULTS.—Extracts from leaves and upper halves of stems, made 24 hours after treatment, caused curvatures of the test plants and later morphological changes of trifoliolate leaves. The extracts from lower halves of the stems failed to cause curvatures but induced morphological changes of trifoliolate leaves. All these extracts gave positive tests with the FREED reagent. There was no evidence of the presence of 2,4-D in the root extracts in either test.

Extracts made 7 days after treatment from the different parts enumerated all induced nastic and formative responses. Also, the color test was positive in all instances.

Likewise, extracts made 26 days after treatment induced in all cases symptoms of the presence of 2,4-D (fig. 2).

The results show that 2,4-D when applied to decapitated stems is translocated in all directions and penetrates into leaves as well as into roots. It can persist in the various organs. Its probable path of movement could be the xylem as well as the phloem and parenchymatous tissue.

Discussion

The results of qualitative tests made to investigate absorption and translocation of 2,4-D are conclusive enough to demonstrate that the chemical when applied in sufficient quantities to intact roots and leaves enters the plant and is translocated beyond the regions of application. The color test used for the detection of 2,4-D in the extracts prepared from treated plants is not entirely specific for 2,4-D, as pointed out by FREED (5). The results, presented by him in tabular form, of the color reactions of a number of organic acids, however, indicate that a wine-purple color is specific for halogen derivatives of phenoxyacetic acid. In other words, regardless of the chemical structure of the substance detected in extracts and exudates from treated plants, it is quite certain that the applied growth-regulator was transported in a form in which the phenoxy ring remained intact. Final evidence as to the actual compound recovered can only be offered after it has been crystallized and its chemical structure determined.

The extracts obtained from treated plants according to the methods described were also physiologically active as evidenced by their induction of nastic and morphological responses in bean plants. These results further substantiate the conclusion that 2,4-D was absorbed and probably translocated as such.

The present investigation on root absorption (with the large quantitites of 2,4-D which were used) and the identification of applied 2,4-D in exudates from

stumps of tomatoes and beans suggest that translocation of the substance from the roots took place mainly with the ascending stream through the xylem. HITCHCOCK and ZIMMERMAN (8), using a similar technique, showed the transport of indoleacetic acid and indolebutyric acid to take place in the same manner. The present findings also support the views of HITCHCOCK and ZIMMERMAN (7), FERRI (4), and MITCHELL and BROWN (10) derived from their indirect evidence.

The root system of the tomato seemed to absorb 2,4-D at a more rapid rate than did those of the other species tested; the substance was therefore translocated in sufficient quantity to be extracted even with water. Obviously the other species absorbed 2,4-D through the roots also and translocated it into the aerial parts, but not in sufficient quantity to be extracted by water.

The translocation of 2,4-D when it is applied to leaves may be through phloem or other living tissues, as suggested by MITCHELL and BROWN (10). The presence of 2,4-D in tomato exudates and the removal of it by aqueous extraction, however, also suggest that at least part of the 2,4-D may have moved through the xylem. SKOOG (11) stated that under special conditions, in the presence of very high concentrations, auxin (indoleacetic acid) introduced into the plant in aqueous solution may also move downward in the xylem. LAIBACH and FISCHNICH (9) suggested that transport may occur in different channels, in xylem, phloem, or parenchyma, depending on conditions and plants.

The results indicate that the method using acid extraction was better suited than aqueous extraction for the separation of 2,4-D from the tissues, probably on account of the hydrolysis performed. The fact that aqueous extracts of corn failed to yield positive tests for 2,4-D is suggestive of a possible combination of 2,4-D with organic substances within the cells from which only a more potent chemical impact can release it.

The movement of aqueous solutions of 2,4-D applied to decapitated plants could have occurred in all kinds of tissues. Part of the growth-regulator probably moved through the xylem, at least in the initial stages. The translocation into the roots, which required some time, may have been effected through living tissues.

The results of the studies further point out that there was multidirectional movement of 2,4-D, because it could be detected in leaves, stems, and roots. CORNS (2) derived similar conclusions from his observations on the killing effect on various organs of sow-thistle plants when 2,4-D was injected into their severed stems.

It was not determined how long a detectable quantity of 2,4-D remained in the tissues after the treatment when the absorption took place through roots and leaves. When the substance was applied to decapitated stems of tomatoes in sufficient quantity, however, it could be found in various organs of the plants even 26 days later.

It is not yet known whether the part of the chemical which reacts with cell constituents is recoverable by the extraction methods used. It is possible that the present method of demonstrating the transport of applied growth substances accounts only for the excess left over after a certain amount of the chemical had reacted within the tissues. When leaves were treated with such limited quantities of 2,4-D as can be applied by means of pipettes or by spraying, and extractions were made of the plants that showed curvatures, the extracts did not contain detectable quantities of 2,4-D.

When the plants were treated by immersing their leaves for 24 and 48 hours, respectively, in rather concentrated solutions, evidently enough of the chemical was taken up to manifest itself even in aqueous extracts. Further work is needed with refined techniques. In addition, it will be necessary to attempt to isolate and identify the chemical from plants which were treated with low concentrations causing physiological responses such as rooting, or into which known quantities of the chemical were introduced. The quantitative recovery of growth-regulators may yield information pertaining to their mode of action. Hypotheses like that of GUTTENBERG could be proved or disproved.

Summary and conclusions

1. Substantial evidence revealed that the roots of tomato, bean, corn, and oat plants are all capable of absorbing the sodium salt of 2,4-dichlorophenoxyacetic acid (2,4-D) from the soil. The absorbed chemical is translocated, probably in its original active form, into the aerial parts but further investigations will be necessary to identify the substance recovered.

2. Attempts to extract and detect 2,4-D failed when it was applied in quantities of a few drops of a 1000-p.p.m. aqueous solution or in equivalent amounts in the form of a paste to leaves of tomato, bean, corn, and oat plants. The immersion of tomato and corn leaves in a 1000-p.p.m. solution of 2,4-D for 24 and 48 hours gave evidence that the growth-regulator is absorbed and proba bly translocated as such. The tissue in which the translocation of the recoverable material takes place is suggested to be the xylem.

3. Following the application of saturated aqueous solutions of the sodium salt of 2,4-D to the cut stumps of decapitated tomato plants, there was a multidirectional movement of the substance, since it was recovered from various organs. The substance was found in extracts of leaves, stems, and roots even 26 days after treatment.

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EFFECTS OF VARIOUS PLANT GROWTH-REGULATORS ON FLOWERING IN SEVERAL CROP PLANTS[1]

ELROY L. RICE

Introduction

During the last 15 years much interest has been evinced concerning the relation of growth-regulators to flowering. The only plant which has been induced to flower almost at will by the application of growth substances is the pineapple (**5, 6, 17, 18**). The majority of workers in this field have reported that plants other than the pineapple are delayed in flowering or prevented from flowering when treated with growth-regulators (**2, 8, 9, 13, 16**). Some workers however, have found slightly earlier flowering in certain plants as a result of treatment with growth substances (**10, 12, 14, 15**). Leopold and Thimann (**11**) stated that the application of minute amounts (approximately 1 μgm. for a 3-week-old plant) of α-naphthaleneacetic acid resulted in an increase of the number of flower primordia produced in Wintex barley, while greater amounts inhibited flowering.

Experiments were devised to test the effect of certain growth-regulators on the flowering of several crop plants, including winter and summer annuals and biennials.

[1] This work was supported in part by a grant from the University of Oklahoma Faculty Research Committee.

Material and methods

The plants investigated included two winter annuals, Wintok oats (C.I. 3424) and Pawnee winter wheat (C.I. 11669); two summer annuals, Little Marvel garden peas and Stringless Green Pod beans; and three biennials, Early Blood Turnip garden beets, Danvers Half Long carrots, and Evergreen white sweet clover. The oat, wheat, and sweet clover seed were furnished through the courtesy of the Agronomy Department of Oklahoma Agricultural and Mechanical College, while the pea, bean, beet, and carrot seed were purchased from Ferry's Seed Company.

The plants were grown in fine sandy loam in field plots on the South Campus of the University of Oklahoma at Norman, Oklahoma, during the growing season of 1949. All were planted in March, with the exception of the beans, which were planted on April 7. The wheat, oats, and clover were planted in strips of four rows each, with rows about 8 inches apart. All other seeds were planted in rows spaced about 40 inches apart.

The plants of each species were divided into nine test groups and one control group. Each test group was sprayed with an aqueous solution of sodium 2,4-dichlorophenoxyacetate [Na(2,4-D)], so-

dium 2,4,5-trichlorophenoxyacetate [Na-(2,4,5-T)], or 4 chloro-o-toloxyacetic acid (4,o-T) in concentrations of 0.1, 1, or 10 p.p.m. The spraying schedule for each species is given in conjunction with each experiment. Na(2,4-D) was obtained through the courtesy of R. W. TOWNE of Monsanto Chemical Company, St. Louis, and Na(2,4,5-T) and 4-o-T through the courtesy of LAWRENCE SOUTHWICK of the Dow Chemical Company, Midland, Michigan.

Experimentation

STRINGLESS GREEN POD BEANS.—The test groups of bean plants were sprayed until thoroughly wetted at the time the second trifoliolate leaf was just starting to expand. The spraying was done on the afternoon of May 5, at which time the sky was partly cloudy and air temperatures ranged from 65° to 83° F. All temperatures were recorded at the University of Oklahoma weather station located 1 mile from the experimental plot, the intervening area being level.

The bean plants sprayed with 0.1-p.p.m. preparations of any of the growth-regulators did not vary appreciably from the corresponding controls in their flowering periods (fig. 1), and those treated with 1-p.p.m. concentrations showed even less variation from the corresponding controls.

The flowering period of bean plants treated with 10-p.p.m. concentrations of 4-o-T coincided quite closely with that of the controls (fig. 2). The beginning of the flowering period was delayed about 2 days in plants treated with the 10-p.p.m. solution of Na(2,4-D) (fig. 2), their peak flowering date was reached 2 days later than that of the controls, and their entire flowering period was prolonged somewhat beyond that of the controls. A suggestion of two periods of peak flowering may be noted from the graph. Plants treated with this concentration of Na(2,4-D) were somewhat stunted, leaf blades were smaller, thickened, wavy, and had prominent veins. The beginning of the flowering period was delayed about 4 days in the plants treated with Na(2,4,5-T) at 10 p.p.m., and their flowering period was prolonged considerably (fig. 2). The date of the peak flowering was 9 days later than in the controls, and there was a pronounced double peak. The period during which flowering was occurring at a fairly rapid rate was much longer in the latter plants than in the controls. Na(2,4,5-T) induced no observable morphological effects on the plants.

Pods were harvested from sixty plants in each test group and controls 1 month after the start of flowering in each group. Using the average total oven-dry weight of pods per plant as a criterion of yield (table 1), there were no statistically significant differences in yield as compared with the corresponding controls despite the pronounced effects of some treatments on the time of flowering.

LITTLE MARVEL GARDEN PEAS.—Pea plants were sprayed first on the afternoon of April 20, when three leaves were expanded and a fourth was just starting to expand. They were sprayed a second time on the afternoon of April 29, when a very few plants were just starting to flower. The sky was clear on both afternoons, with air temperatures ranging from 50° to 66° F. on April 20 and from 57° to 73° F. on April 29. The amount of each solution applied was equivalent to approximately 70 gallons per acre at each spraying. No spreading agent was added to any sprays at any time and, consequently, the plants were not thoroughly

wetted, even though the amount of spray solution applied was sufficient to do so if all of it had adhered to the plants.

Flowering began at the same time in sprayed plants as in the controls, reached a peak at the same time, and ceased at essentially the same time. There were no outward morphological effects on leaves resulting from any treatment. The pods were considerably distorted, however, on plants sprayed with 10-p.p.m. concentrations of Na(2,4-D).

Mean dry weight of pods per plant in each treatment was determined. The only significant data (table 2) indicate that the treatments caused slight reductions in yield.

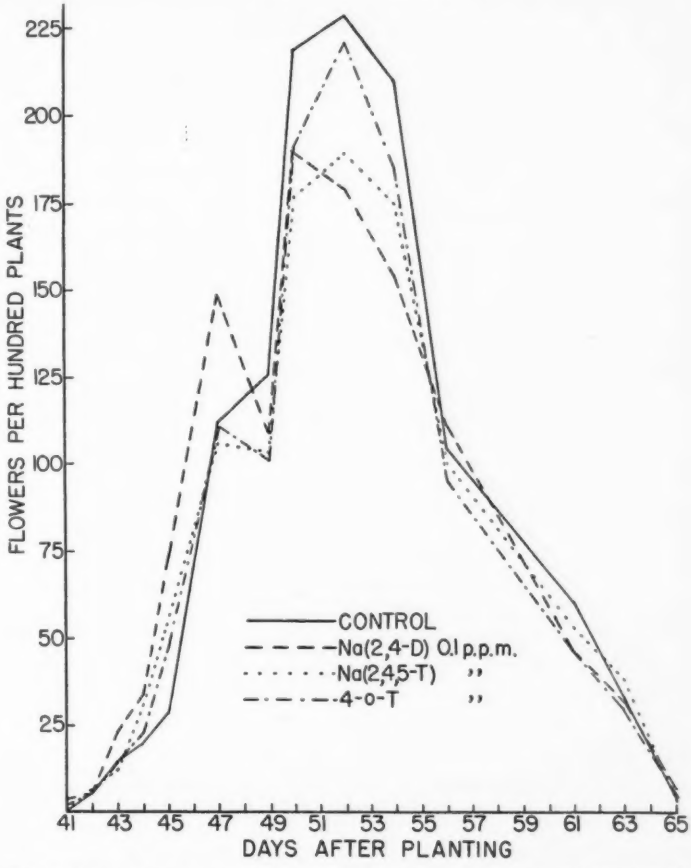

FIG. 1.—Effect of 0.1-p.p.m. sprays of various growth-regulators on time and quantity of flowering of Stringless Green Pod beans.

PAWNEE WINTER WHEAT.—The fact that many winter annuals such as winter wheat and winter rye will not flower unless exposed to a certain minimum number of hours of low temperatures is well known. In the present experiment the winter wheat was planted in the spring

to see if it would be induced to flower by the application of the previously mentioned growth-regulators without first having been exposed to the low temperatures of the winter months. The planting

In addition to the ten regular plots, three extra plots were used. The latter were sprayed twice with 0.1-p.p.m. concentrations of the growth-regulators within a 9-day period.

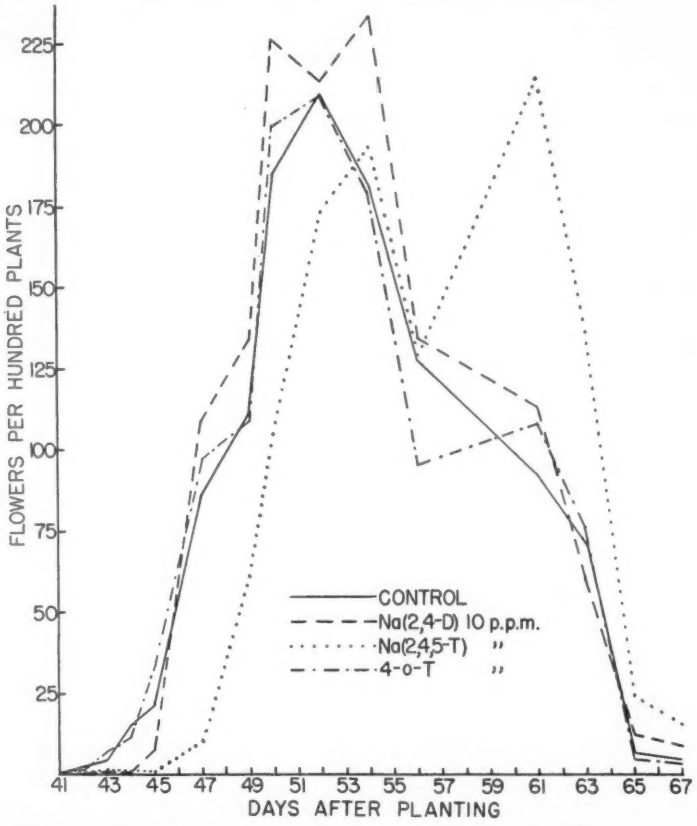

FIG. 2.—Effect of 10-p.p.m. sprays of various growth-regulators on time and quantity of flowering of Stringless Green Pod beans.

date was March 16; after that date the minimum daily temperatures were slightly below freezing on only 2 days, March 19 and 20. There were only 7 days with minimum temperatures below 40° F. after March 20, and, with the exception of 4 days, the maximum daily temperatures were in the sixties or higher.

The wheat plants were sprayed first on the afternoon of April 12, at which time the sky was clear and air temperatures ranged from 42° to 68° F. Most of the plants then had three leaves with expanded blades. The three extra plots were sprayed a second time on April 20, on which date air temperatures ranged

from 50° to 66° F. The approximate rate of applications was 70 gallons per acre at each spraying.

The nine regular treated plots were sprayed again on the afternoon of June 11 at the approximate rate of 100 gallons per acre. The sky was clear, and temperatures ranged from 63° to 86° F. For 3 weeks before this second spraying the wheat showed symptoms of a heavy infestation of orange leaf rust. This disease may have had considerable effect on the results of the experiment.

No flowering (anthesis) occurred in any of the wheat plants, treated or not, nor did any exsertion occur. In fact, there was practically no culm elongation in any of the plants. Culms were collected from ten or more plants in each of the thirteen plots on July 14 and July 21. Dissection under a microscope revealed that the majority of shoot apices from all plots were in a transition stage toward the development of inflorescences as recognized by the occurrence of primordia with double ridges (3). Most of the wheat plants were dead by about August 1.

TABLE 1

EFFECT OF VARIOUS GROWTH-REGULATORS ON YIELD OF STRINGLESS GREEN POD BEANS. EACH VALUE BASED ON SIXTY-PLANT SAMPLE

TREATMENT	MEAN DRY WEIGHT (GM.) OF PODS PER PLANT*	
	0.1 p.p.m.	10 p.p.m.
Controls	13.6	14.4
Na(2,4-D)	12.3	15.7
Na(2,4,5-T)	11.3	14.2
4-o-T	12.2	15.1

* Differences from corresponding controls not significant.

WINTOK OATS.—Since this variety may be planted in either fall or spring in Oklahoma, it is not, in a strict sense, a true winter annual.

In this experiment the planting and spraying schedule was the same as that for the wheat except that the nine regular test plots were not sprayed a second time. There were no apparent differences in time of flowering or amount of flowering in any of the sprayed plants as compared with the controls.

TABLE 2

EFFECT OF VARIOUS GROWTH-REGULATORS ON YIELD OF LITTLE MARVEL GARDEN PEAS. VALUES BASED ON SAMPLES OF 26–89 PLANTS

TREATMENT (P.P.M.)	MEAN DRY WEIGHT (GM.) OF PODS PER PLANT		
	Na(2,4-D)	Na(2,4,5-T)	4-o-T
0	4.0	5.0	5.2
0.1	2.9*	5.4	4.4
1	4.9*	4.1*	4.3*
10	3.5	4.0*	5.6

* Difference from corresponding controls significant at 5% level or better.

BIENNIALS.—The sweet clover, beets, and carrots were planted in March, and each was sprayed a total of three times during the spring and summer months with the three concentrations of each of the three growth-regulators. No plants showed any outward morphological effects after any spraying. Moreover, none of the plants flowered during the first growing season.

Discussion

In no case in the present investigation was there a stimulation of earlier flowering resulting from the application of any concentration of any of the growth-regulators tested. On the other hand, there was a pronounced delay in flowering of those bean plants sprayed with 10-p.p.m. concentrations of either Na(2,4-D) or Na(2,4,5-T), the latter resulting in the greatest delay. Plants treated with the former

substance were somewhat deformed and stunted, so it is possible that the delay in flowering could have been due to a slowing of growth rather than to a direct effect on differentiation of flower primordia. If true, this would be in agreement with the conclusion of LEOPOLD and THIMANN (11) that auxin does not simply oppose flowering but exerts an effect analogous to its effect on vegetative development. On the other hand, growth of plants treated with a 10-p.p.m. concentration of Na(2,4,5-T) was apparently not affected in any way. This suggests that the latter substance had a direct effect on differentiation of flower primordia. Results of greenhouse trials on Bountiful beans (9) and on Red Kidney beans (13) were almost identical with those obtained in the present field investigation. In field experiments, however, STROMME and HAMNER (13) reported that 1-p.p.m. and 10-p.p.m. concentrations of Na(2,4-D) had no effect on the time of flowering of Red Kidney beans. With a higher concentration (100 p.p.m.), however, the latter workers found that flowering was markedly delayed.

No report has been found concerning the effect of 2,4,5-T on flowering in any plants. Its pronounced effect in delaying flowering of Stringless Green Pod beans without bringing about any outward morphological changes or decreases in yield suggests a need for further research along this line.

The results of the present investigation agree with those of the majority of workers that the application of growth substances generally brings about a delay in flowering rather than an induction of flowering in the plants investigated. In fact, according to CHOLODNY (4), DOSTAL and HOSEK found that "flower ready" stem tips of *Circaea* could be made to revert to the vegetative habit by the application of auxins. In the light of all the evidence which is accumulating it would seem more logical to use an anti-auxin, or perhaps an auxin inhibitor, in attempts to induce flowering. This is at least partially borne out by the recent work of BONNER (1), who found that the application of the anti-auxins 2,4-dichloranisole and triiodobenzoic acid, to *Xanthium* plants kept on long photoperiods resulted in the initiation of flower-like buds in treated plants, while untreated plants remained vegetative. Moreover, GALSTON (7) found that the flowering response of Peking and Biloxi soybean plants was augmented by the application of triiodobenzoic acid.

Summary

1. An investigation was made of the effects of sprayed aqueous solutions of sodium 2,4-dichlorophenoxyacetate [Na-(2,4-D)], sodium 2,4,5-trichlorophenoxyacetate [Na(2,4,5-T)], and 4 chloro-o-toloxyaceticacid [4-o-T] in concentrations of 0.1, 1, and 10 p.p.m. on flowering and yield of winter annuals (Wintok oats and Pawnee wheat), summer annuals (Little Marvel garden peas and Stringless Green Pod beans), and biennials (Early Blood Turnip garden beets, Danvers Half Long carrots, and Evergreen white sweet clover).

2. No treatment was successful in inducing flowering in nonvernalized winter wheat or in inducing flowering in the biennials during the first growing season.

3. No differences in time or amount of flowering of oats were observed in any treatments.

4. The sprays had no effect on time or quantity of flowering in peas. The only statistically significant effect on yield of peas was a slight reduction.

5. Treatments with 0.1- or 1-p.p.m. concentrations of each growth-regulator or with 10 p.p.m. of 4-o-T appeared to have little or no effect on the time of flowering of the beans. Bean plants sprayed with 10-p.p.m. solutions of Na(2,4-D) or Na(2,4,5-T) were delayed in flowering by 2 days and 4 days, respectively. The peak flowering date was delayed 9 days by the latter treatment. In the former case the plants showed pronounced morphological effects, but in the latter case no outward morphological changes were evident. None of the treatments had any significant effect on the yield of beans.

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EFFECTS OF DIFFERENCES IN PHOSPHORUS NUTRITION ON THE NITROGEN CONTENT AND PHOSPHORUS 32 ABSORPTION OF FOUR SPECIES OF GRASS

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Introduction

This paper is a report on the effects of different levels of phosphorus nutrition on nitrogen content and phosphorus 32 absorption in four species of grass: *Andropogon ischaemum* L., the King Ranch bluestem strain; *Bouteloua curtipendula* (Michx.) Torr., side-oats grama; *Eragrostis trichodes* (Nutt.) Wood, sand lovegrass; and *Sorghastrum nutans* (L.) Nash, Indian grass. These species are perennial grasses of some economic importance in the southern United States. All are of upright habit, a factor of concern when experiments must be conducted in greenhouse cultures. Each has certain features of particular interest with respect to the relationships between physiological characteristics and developmental characteristics.

Material and methods

Seeds of all four species were kindly furnished by the San Antonio nursery of the Soil Conservation Service of the United States Department of Agriculture. The seeds were soaked in distilled water for 6 hours, sown in acid-washed quartz sand in 1-gallon glazed crocks, and covered with approximately ½ inch of sand. Immediately before sowing the seeds, 500 ml. of WOLKOFF's (17) modification of Shive's solution were added to each crock. Trace-element solutions A and B of HOAGLAND and ARNON (6) were used with the nutrient solution. Crocks containing the different species were distributed on the greenhouse bench in a randomized block pattern. During germination and early seedling growth the crocks were covered with glass plates so as to maintain a high moisture level.

During the first 2 weeks after emergence the seedlings were watered every other day with the complete nutrient solution. At the end of this period four crocks of each species (designated hereafter as Series A) were deprived of any further supply of phosphorus by substituting a phosphorus-free nutrient solution in which KCl replaced KH_2PO_4. Series B, C, D, and E, of like composition, were similarly deprived 3, 4, 5, and 6 weeks, respectively, after emergence. The control, Series N, was watered twice a week throughout the entire 7-week experimental period with the complete nutrient solution (500 ml. each time).

At the beginning of the experiment there were approximately 200 seedlings per crock, this number being gradually reduced during sampling. At frequent intervals plants were removed for morphological studies and evaluation of growth, a discussion of which will appear in a separate publication. In sampling, an attempt was made to keep the number of plants per crock approximately uniform, and any atypical or injured plants were eliminated. Sampling for the phosphorus studies was begun 7 weeks after emergence. Five plants of each species from each series were transferred from the sand cultures to distilled-water cultures preliminary to the feeding of radiophosphorus. This transfer was effected by emptying the crocks and carefully separating the roots from the sand by wash-

ing with water. The plants were then inserted in perforated corks, and their roots were immersed in distilled water in 1-quart Mason jars. To provide for recovery from possible injury, the roots were allowed to remain in the water for 1 week. At the end of the recovery period phosphorus 32 in the form of orthophosphoric acid was added to the water. The initial radioactivity of this solution was about 10 μc/ml of solution.

The plants with their roots in the radiophosphorus solution were allowed to stand in the greenhouse during the absorption period. A preliminary experiment with *A. ischaemum* had indicated that the absorption of P^{32} under such conditions is a relatively slow process. Hence, the experimental plants were left with their roots in the solution for 26 hours (from 12:00 M. until 2:00 P.M. the following day). They were then removed from the jars, and their roots were washed in running water. Shoots and roots were dried and weighed separately. The samples were then ground in a Wiley mill to pass through a 60-mesh screen. The mill was cleaned carefully after the grinding of each individual sample. The finely ground powder was kept in separate paper envelopes in desiccators until analyses could be made.

Each sample was analyzed for Kjeldahl nitrogen, total phosphorus, and total radiophosphorus. For Kjeldahl nitrogen a direct Nesslerization of acid-digested mixtures was followed by photocolorimetric estimation by the method of KOCH and MCMEEKIN (7). Four-milligram samples were weighed into large Pyrex test tubes which had been calibrated to 10 ml., and 3 ml. of 1:1 H_2SO_4 were added to each test tube. A few drops of 30% H_2O_2 were added when the contents of the tubes were almost clear. After digestion was complete, distilled water was added up to the 10-ml. mark. Ten milliliters of the Nessler-Folin solution (Koch modification) were then added to develop color, and readings were taken through a green filter 10 minutes later. A standard curve was prepared by using a series of dilutions of the standard solution of NH_4Cl. For the determination of total phosphorus, the procedure of FISKE and SUBBAROW (5) was followed after the plant material had been ashed by the method of WOLF (16). Radioactivity of the samples of dry powder was determined with a Geiger-Müller radioactivity counter apparatus. In each instance two counts were made on each of the duplicate 20-mg. samples. After the first count of each sample the powder was redistributed in the aluminum sample pan, and a second count was made. Final radioactivity figures were obtained by averaging the counts made on each set of duplicate samples.

Results

The average dry weights of roots, shoots, and whole plants after 7 weeks' growth in sand culture and 1 week in water culture are shown in table 1. In evaluating the data for root weight, it should be borne in mind that there may have been some loss of root tissue in the transfer of the plants from sand to water in preparation for the feeding of radioactive phosphorus. In each species, treatment-A plants, which were fed phosphorus for only 2 weeks, made distinctly less shoot growth than the plants in any of the other treatments. The longer phosphorus feeding periods did not give any consistent advantage in shoot growth.

The total phosphorus content of the shoots on the basis of μgm/mg of dry weight is given in table 3. Except in the case of *B. curtipendula* the absorption of phosphorus was apparently continued

throughout the experimental period. The four species differed significantly in the amount of phosphorus absorbed. At the end of the 7-week period the *S. nutans* shoots had accumulated nearly three times as much phosphorus per unit of dry weight as had those of *B. curtipendula*.

The percentage values of Kjeldahl nitrogen on a dry-weight basis are given in table 2. The nitrogen contents were lower than have been reported by some investigators (10), but this may be the result of sampling differences or of seasonal differences. There was not a consistent nitrogen percentage increase with age.

Table 3 gives data on the amount of P^{32} absorbed, on the basis of counts per minute per 20 mg. of dry weight. These results are given for shoots only. Although root samples were counted, the counts were so high and so variable, probably as the result of differences in the amount of radioactive phosphorus retained on the root surfaces, that the results did not seem valid and have therefore been omitted. In all four species the amount of radiophosphorus absorbed increased with the length of time over which the plants had been fed phosphorus. These data, considered with those on total phosphorus, show clearly that the longer the period over which the plants were fed phosphorus, the greater was the accumulation of both total phosphorus and radiophosphorus.

The data on P^{32} absorption expressed as counts per unit microgram of total phosphorus in the tissue (table 3) show an increase in the amount of P^{32} with each progressively longer phosphorus-absorbing period. This fact suggests a definite relationship between the amount of P^{32} absorbed and the amount of phosphorus previously present in the tissue.

TABLE 1

DRY WEIGHTS IN GRAMS (AVERAGE OF THREE PLANTS) AFTER 7 WEEKS IN SAND CULTURES AND 1 WEEK IN WATER (SEE TEXT)

Treatment	*A. ischaemum*			*B. curtipendula*			*E. trichodes*			*S. nutans*		
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
A	0.25	0.86	1.11	0.17	0.39	0.56	0.10	0.25	0.35	0.14	0.33	0.47
B	.31	1.45	1.76	.07	.46	.53	.21	.54	0.75	.12	.70	0.82
C	.31	1.14	1.45	.08	.47	.55	.05	.36	0.41	.22	.90	1.12
D	.25	1.85	2.10	.12	.52	.64	.18	.57	0.75	.28	.90	1.18
E	.27	1.21	1.48	.13	.47	.60	.15	.41	0.56	.17	.77	0.94
N	0.24	1.43	1.67	0.13	0.56	0.69	0.19	0.83	1.02	0.24	0.53	0.77

The data for absorption of P^{32} on a per-plant basis (table 3) indicate approximately a tenfold increase of such absorption when the phosphate-feeding period was increased from 2 to 7 weeks.

The absorption and distribution of P^{32} in *A. ischaemum* was also evaluated by the radioautograph technique. Four-week-old plants were transferred from the sand cultures to a nutrient solution rather than water for absorption prior to radioautography. After a recovery period of 24 hours P^{32} was added to the nutrient solution. After a 4-hour absorption period the plants were removed and their roots washed thoroughly with tap water. The roots were dried by blotting with filter paper, and the plants were then

allowed to dry in a herbarium press. Radioautographs were made by pressing dried plants on Agfa Non-Screen X-ray films in light-tight boxes for a sufficient gregation of the leaf sheaths in this region. The work of RABIDEAU *et al.* (13) has indicated clearly, however, that the P^{32} is concentrated in the shoot apical

TABLE 2

PERCENTAGE OF KJELDAHL NITROGEN ON DRY-WEIGHT BASIS

TREATMENT	SHOOT				ROOT			
	A. ischaemum	*B. curtipendula*	*E. trichodes*	*S. nutans*	*A. ischaemum*	*B. curtipendula*	*E. trichodes*	*S. nutans*
A	1.50	1.50	1.00	1.30	0.86	1.25	1.00	1.26
B	1.74	1.26	1.00	1.70	0.90	1.26	1.34	1.34
C	1.70	1.26	0.50	1.50	1.00	1.26	1.16	1.40
D	1.74	1.50	0.52	1.50	1.10	0.96	1.16	1.16
E	2.00	1.26	1.00	1.70	0.90	0.84	1.34	1.26
N	2.00	1.00	1.00	2.15	0.80	0.84	1.35	0.86

TABLE 3

TOTAL PHOSPHORUS AND PHOSPHORUS 32 IN SHOOTS

SERIES	*A. ischaemum*	*B. curtipendula*	*E. trichodes*	*S. nutans*	*A. ischaemum*	*B. curtipendula*	*E. trichodes*	*S. nutans*
	Total phosphorus (μgm/mg dry wt.)				P^{32} absorbed (counts/min/20 mg dry wt.)			
A	1.4	0.8	0.7	2.1	159	110	482	219
B	1.5	0.9	0.7	2.4	424	186	506	339
C	1.5	1.7	1.1	2.8	605	240	816	632
D	1.8	1.7	1.3	3.1	726	316	825	1186
E	2.5	1.3	1.8	4.1	928	552	966	1353
N	3.1	1.5	2.2	4.2	1065	919	970	1668
	P^{32} absorbed (counts/μgm total phosphorus)				P^{32} absorbed on a per-plant basis (counts/min/plant)			
A	5.87	6.87	34.29	5.21	2,942	1,034	2,820	2,049
B	14.68	10.33	36.15	7.06	12,338	1,590	6,330	4,580
C	20.24	13.62	37.09	11.28	14,587	2,280	5,671	11,581
D	24.20	9.29	31.73	19.18	25,446	3,365	10,341	23,356
E	18.55	21.72	26.84	16.50	22,923	5,492	7,984	21,294
N	17.18	30.62	22.05	21.05	29,526	10,603	16,490	21,267

number of hours to permit accumulation of the required amount of radiation. Figure 1 shows the distribution of radiophosphorus in a typical plant, with most P^{32} in the region surrounding the shoot apical meristem. This concentration is undoubtedly in part a result of the ag- meristems of actively growing corn plants; it can be assumed that the apparent concentration in *A. ischaemum* is in part an actual concentration in the meristematic region.

The relationships of nitrogen content, total phosphorus content, and P^{32} ab-

sorption are presented in figure 2. There is obviously a definite relationship between total phosphorus and the absorption of P^{32}. The graphs fail to reveal any direct relationship between the level of phosphorus nutrition and the nitrogen content of the plants.

FIG. 1.—Radioautograph of plant of *Andropogon ischaemum* showing distribution of P^{32} after 4-hour absorption period.

Discussion

Growth of all four species of grasses, as measured by dry weight, increased with the continued addition of phosphorus to the medium. It is significant, however, that, in general, plants fed phosphorus for 3 or 4 weeks made as much or nearly as much growth as those fed phosphorus for longer periods. This fact emphasizes a difficulty in attempting to determine the phosphorus needs of plants by "foliar diagnosis."

In discussing the quantitative mineral-nutrient requirements of plants, MACY (**11**) proposed a theory of the relationship between the sufficiency of a nutrient and its percentage content in the plant. He stated: "The central concept is a *critical percentage* of each nutrient in each kind of plant, above which there is a *luxury consumption*, and below which there is *poverty adjustment*, which is almost proportional to the deficiency until a minimum percentage is reached." The results of the present work support MACY's hypothesis.

No marked decrease in the ratio of shoot weight to root weight was noted. This would seem to suggest that the growth of roots was not particularly stimulated by the continued availability of phosphorus. TURNER (**15**) has suggested that higher phosphate concentrations tend to favor root growth, while SOMMER (**14**) failed to find such an effect. It is to be supposed, however, that differences in experimental methods and differences among species would result in diverse findings with respect to such a consideration.

An inverse relationship between phosphorus and nitrogen absorption has been reported by various workers (**4, 8, 9, 12**) working with tomato plants in water cultures. ARNON and HOAGLAND (**1**), however, failed to note any change in nitrogen percentage of tomato leaves or fruit with the change in phosphorus supply in the medium. They attributed this lack of change to the fact that the medium contained a liberal supply of nitrogen. The results obtained in the present experiments also fail to reveal an appreciable

change in nitrogen percentage with the change in phosphorus supply and may likely be explained on the same basis as the results of ARNON and HOAGLAND (1). phorus-deficient and the control plants, respectively, 3.8 and 7.8% in a 4-hour migration period; for an 8-hour migration the comparable figures were 11.1

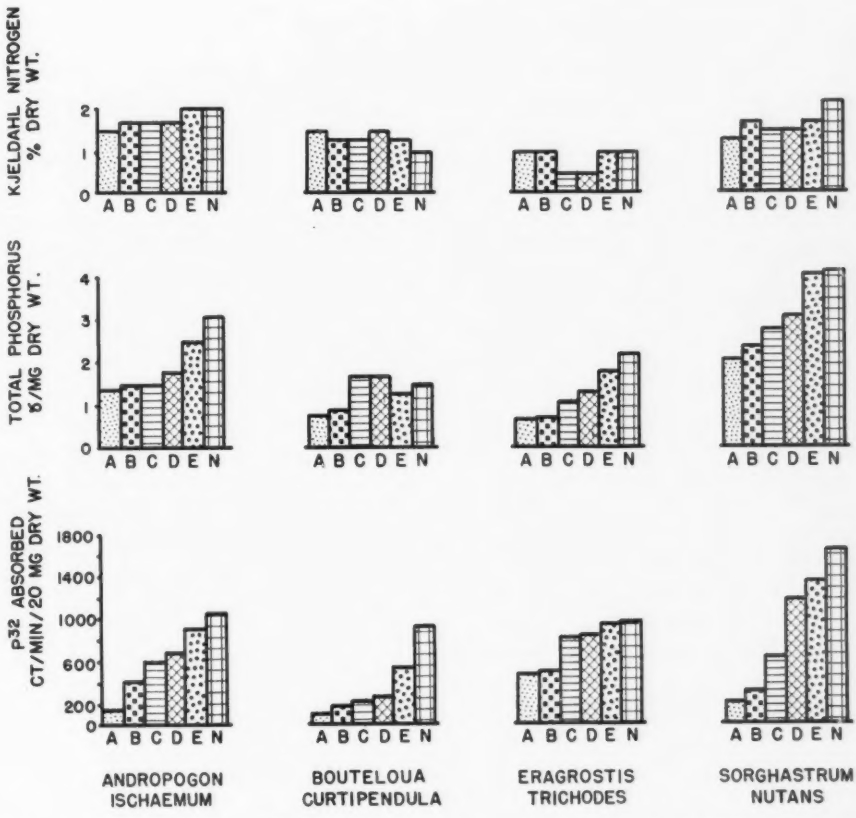

FIG. 2.—Histograms showing Kjeldahl nitrogen on a percentage dry-weight basis, total phosphorus on a γ/mg of dry-weight basis, and the amount of P^{32} absorbed in terms of counts per minute per 20 mg. of dry weight in shoots of *Andropogon ischaemum*, *Bouteloua curtipendula*, *Eragrostis trichodes*, *Sorghastrum nutans*. The letters *A*, *B*, *C*, *D*, *E*, and *N* refer to various treatments.

BIDDULPH (2), in studying the various factors affecting the migration of injected P^{32} in bean plants, reported that phosphorus deficiency retarded the migration. When phosphorus was omitted from the nutrient solution during a 2-week period preceding the experiment, the injected P^{32} migrated in the phos- and 16.3%. BIDDULPH therefore suggested that, among other possible factors such as transpiration and sugar metabolism, the phosphorus content of the tissue might exert a controlling influence on the migration. The present investigation clearly demonstrates the dependence of P^{32} absorption on the phosphorus con-

tent of the tissue. On a per-plant basis, in all four species, plants which were supplied with phosphorus for 7 weeks absorbed from seven to ten times as much P^{32} as plants which were supplied with phosphorus for only 2 weeks (table 2). The fact that the figures for counts of P^{32}/P^{31} (table 3) do fluctuate within each species seems to suggest, however, that the phosphorus content of the tissues was not the only factor determining the uptake and migration. Part of the difference can be explained as being related to the larger size of the plants fed for the longer periods. There are presumably still other factors involved.

BREON *et al.* (3) reported that phosphorus-deficient tomato plants absorbed P^{32} at a higher rate than control plants fed adequate amounts of phosphorus. The findings in the present experiment differ greatly from their results. No explanation of the differences between the work of these investigators and ours is at hand, but the conditions of this experiment were quite different, and it can be assumed that grass plants may not necessarily react in the same manner as do tomato plants.

Summary

1. Plants of four species of grass, *Andropogon ischaemum*, the King Ranch bluestem strain; *Bouteloua curtipendula*, side-oats grama; *Eragrostis trichodes*, sand lovegrass; and *Sorghastrum nutans*, Indian grass, were grown from seed in sand culture and provided with available phosphorus over periods varying from 2 to 7 weeks. Sample plants were then fed P^{32} by the root-absorption method. Subsequently each sample was analyzed for Kjeldahl nitrogen, total phosphorus, and total radiophosphorus.

2. In all four species plants fed phosphorus for only 2 weeks made significantly less growth than those fed for longer periods. Because of the variations involved and the fact that all four species are perennials, no conclusions can be drawn concerning the relationship between later phosphorus absorption and total growth. In general, plants fed phosphorus for periods of from 3 to 7 weeks did not make much greater growth than those fed phosphorus for only 2 weeks. In every case, however, the absorption of phosphorus continued throughout the experimental period.

3. There were distinct differences among the species in the amount of phosphorus accumulated on a dry-weight basis. The amount of P^{32} absorbed appeared to be conditioned by the amount of phosphorus already in the tissues, those plants containing the greatest amount of phosphorus absorbing the greatest amounts of P^{32}. These plants were also the largest.

4. The inverse relationship reported by others between phosphorus and nitrogen absorption does not find support in the data reported here. This may be a result of the fact that the nitrogen supply was purposely maintained at a fairly high level throughout the experiment.

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COTTON-LEAF MALFORMATIONS INDUCED BY ORGANIC PHOSPHATE INSECTICIDES[1]

WAYNE J. MCILRATH

Introduction

During the spring of 1949 leaf abnormalities similar to those resulting from application of 2,4-dichlorophenoxyacetic acid (2,4-D) were pointed out to the author on cotton plants which had been treated with a hexaethyl tetraphosphate insecticide formulation. Although ZIMMERMAN and HARTZELL (9) reported that when hexaethyl tetraphosphate and tetraethyl pyrophosphate were applied to numerous plant species no hormone-like responses were observed, other workers (1, 6, 7) have reported such responses after prolonged application of these materials. It was uncertain in this case, therefore, whether the responses were due to the insecticide or to 2,4-D–contaminated spray equipment. To check this, greenhouse-grown cotton plants were treated with a single application of a wide range of aqueous dilutions of this insecticide. At certain dilutions it was

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found that the formative effects again appeared. This investigation was therefore undertaken to determine the effects of various dilutions of several commercial formulations of three organic phosphate insecticides: namely, hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP) and O,O-diethyl O-p-nitrophenyl thiophosphate (parathion). Thirty-day-old Stoneville 2B cotton was utilized as the test plant throughout the investigation.

Experimentation

HETP AND TEPP.—With single applications of the HETP and TEPP formulations the plant response varied with the concentration from no noticeable effect to complete killing. As a general rule, aqueous-solution concentrations in excess of fifty times that recommended for insecticidal application resulted in extreme burning of the foliage and/or death of the plant; concentrations approximately fifteen to thirty times that recommended caused slight foliage burning and malformation of subsequently developed leaves; and up to ten times the recommended level, no visible effects. At the dilutions which caused extreme burning of the foliage no formative effects were ever observed to result. In the range causing formative effects, both HETP and TEPP induced leaf malformations very similar to that resulting from 2,4-D (fig. 1). SMITH (5) has stated that new growth of tomato plants following HETP spray also exhibited 2,4-D–like injury. HALL (4) reported a similar response for tomato, sunflower, and carnation plants and indicated that these species are very sensitive to these substances, lethal effects being obtained at much lower dilutions than those indicated above.

The severity of malformation of individual leaves produced subsequent to treatment followed a pattern very similar to that noted when cotton was treated with small quantities of 2,4-D (unpublished data) or that indicated for Red Kidney bean by WATSON (8). With certain dilutions of HETP it was observed that malformed leaves were present at as many as ten nodes. It was also found that leaves at as many as four of these nodes were malformed as severely as illustrated in figure 1. Such severity of malformation was not observed following any of the applications of TEPP.

When equivalent dilutions (based on active insecticidal ingredient) of the HETP and TEPP formulations were applied, the HETP caused more severe leaf malformations. The reason for this is unknown, although several possibilities seem to exist. Since it has been demonstrated that the HETP in insecticidal formulations is probably not a definite compound but rather a mixture of esters containing ethyl metaphosphate, triethyl orthophosphate, tetraethyl pyrophosphate, and possibly pentaethyl triphosphate (3), the additive effect of several organic phosphates may account for the greater degree of malformation resulting from this material. Although it has been shown that triethyl orthophosphate and ethyl metaphosphate as well as monoethyl and diethyl orthophosphate (hydrolytic products of HETP) are relatively nontoxic to insects (3), the possibility that they are active in inducing plant malformations cannot be excluded without further experimentation. In this investigation it was observed that no formative effects resulted from applications of aqueous sprays of triethyl phosphate. It was also found, however, that when TEPP (98.1% purity) was applied to plants in quantities several times that present in the HETP dilutions, the resultant malformations were much less

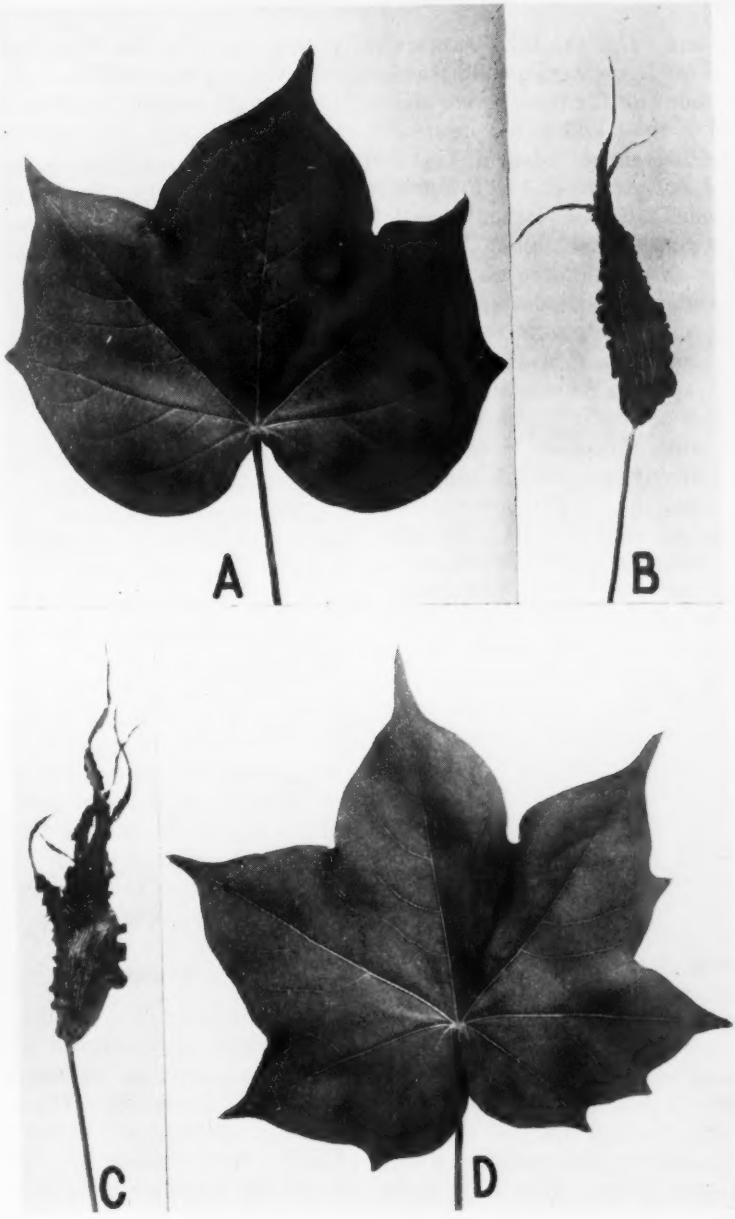

FIG. 1.—Cotton leaves. *A*, normal leaf from untreated plant; *B*, from plant treated with 2,4-D; *C*, from plant treated during the winter months with formulation of hexaethyl tetraphosphate, thirty times the recommended insecticidal dosage; *D*, from plant treated with parathion, three times the recommended dosage.

severe than when the HETP formulation was used. Another possibility which may account for the more severe effects induced by the HETP is the presence of some undetermined substance in this material, not present in TEPP formulations, which facilitates formative action of the organic phosphates.

It was observed during the course of this investigation that the season of the year at which the plants were treated played an important role in the severity of the injury. The most severe leaf malformations appeared during the winter months when the plants were growing under relatively low light intensities and temperatures (fig. 1). This observation is in agreement with that made by other workers (**1, 2, 5, 6, 7**). The importance of the light-intensity factor was demonstrated by treating plants grown during the summer months (temperatures of 75°–98° F.) under high and low light intensities with HETP. The leaf malformations exhibited by plants grown under low light intensity were much more severe than those shown by plants grown under high light intensity and were as severe as the malformations exhibited by plants grown in the greenhouse during the winter months (fig. 1). To check the influence of temperature, summer-grown greenhouse plants were segregated into two temperature treatments at the time of spray applications: namely, 80°–105° F. and 60°–80° F. This latter temperature range closely approximated that present in the greenhouse during the winter months. Plants at the lower temperatures exhibited malformations which were more severe than those at the higher. It is possible that this difference may be accounted for by the more rapid hydrolysis of the active organic phosphates at the higher temperature (**3**), with their period of activity thus being shorter. The relative importance of light intensity and temperature varied somewhat with the formulation.

It was determined that the severe foliage burning resulting from applications of the TEPP formulations was due not primarily to this compound but rather to the other organic phosphates present. Triethyl phosphate was very active in inducing such foliage burning at high temperatures (80°–100° F.) and to a lesser extent at lower temperatures (60°–80° F.).

PARATHION.—The application of parathion to plants in high dosages resulted in a different type of leaf response from that induced by HETP and TEPP. With this material leaves developed subsequent to treatment tended to be abnormal only in being many-lobed rather than having the usual number of three to five lobes (fig. 1). Plants which exhibited severe foliage burning owing to parathion treatment did not show this increased leaf lobing in the new growth. The increased lobing was induced by parathion in an aerosol with a concentration as low as three times the recommended insecticidal dosage. The severity of this abnormality did not seem to vary with season.

Summary

1. This investigation dealt with the leaf responses of 30-day-old Stoneville 2B cotton plants to various concentrations of hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), and O,O-diethyl O-p-nitrophenyl thiophosphate (parathion).

2. Application of commercial formulations of HETP and TEPP in concentrations of fifteen to thirty times the recommended insecticidal dosage resulted in

malformation of leaves subsequently produced. The malformation closely resembled that induced by 2,4-D.

3. HETP was more effective in inducing such leaf malformations than was TEPP.

4. Plants grown during the winter months exhibited more severe leaf malformation than those grown in the other seasons. The factors of light intensity and temperature are discussed in this regard.

5. Parathion applications resulted in the production of leaves which were many-lobed as contrasted to the three to five lobes of normal leaves.

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EFFECT OF COCONUT MILK ON THE GROWTH IN VITRO OF PLANT VIRUS TUMOR TISSUE

LOUIS G. NICKELL

Introduction

During the past decade many workers have found that coconut milk (the fluid endosperm from *Cocos nucifera*) stimulates the growth *in vitro* of various plant materials, including embryos of the Jimson weed (*Datura*) (**13, 14**) and normal tissues from carrot (*Daucus carota*) (**4**), Jerusalem artichoke (*Helianthus tuberosus*) (**5**), Boston ivy (*Parthenocissus tricuspidata*) (**6**), nasturtium (*Tropaeolum majus*) (**1**), and white lupine (*Lupinus albus*) (**1**). Recently, DUHAMET found that coconut milk stimulates the growth *in vitro* of crown-gall tissue from black salsify (*Scorzonera hispanica*) (**7**). He stated that this is the first example of a substance capable of stimulating the proliferation of a tumorous tissue, since indoleacetic acid does not stimulate the growth of this crown-gall material (**8, 9**). In view of this recent report, it was

thought desirable to present data concerning the effect of coconut milk on the growth *in vitro* of virus tumor tissue from sorrel (*Rumex acetosa* L.).

Material and methods

The tissue used was the original R_1 strain isolated by BLACK (2), and the unautoclaved (Seitz sterile-filtered) at the different levels shown in table 1 and was also used as pure coconut milk with 1% Difco Noble agar added.

All experiments were run in replicates of five and an average taken. The duration of the growth period was 3 weeks.

TABLE 1

GROWTH RESPONSES OF VIRUS TUMOR TISSUE FROM "RUMEX ACETOSA" ON BASAL MEDIUM (NO. 24) "IN VITRO" TO ADDITION OF AUTOCLAVED AND UNAUTOCLAVED (FILTERED) COCONUT MILK AND TO PURE COCONUT MILK

SUBSTANCE ADDED	AMOUNT, ML/ML MEDIUM	RELATIVE COLOR*		GROWTH VALUE†
		Green	Brown	
Control (no. 24)	.....	5	0	5.3
Coconut milk (unautoclaved)	0.1	4	0	5.6
	0.2	5	0	6.8
	0.3	5	0	6.8
	Pure	5	1	8.4
Coconut milk (autoclaved)	0.1	6	0	8.6
	0.2	6	0	9.6
	0.3	6	0	11.1
	Pure	6	0	9.4

* For a discussion of relative color see BURKHOLDER and NICKELL (3).

† Growth value is a figure obtained by dividing the final wet weight by the weight of the original inoculum.

cultural conditions were the same as those maintained in previous studies with this material (3). The basal medium was no. 24 of NICKELL and BURKHOLDER (3, 11) on which all stock cultures have been grown for the last 3 years.

The coconut milk was obtained from young, immature coconuts in the "jelly" stage, i.e., with the copra still in a gelatinous state just within the shell (15). The nuts were collected in southern Florida and generously supplied by Dr. V. M. CUTTER. The milk was added to the basal medium both autoclaved and

Results

Coconut milk is stimulatory to the growth of the virus tumor tissue in all concentrations added (table 1). Autoclaved milk is much more stimulatory than the milk which has been sterile-filtered, and pure milk (with 1% agar) will itself support better growth than that on the basal medium. This last fact indicates that coconut milk is capable of supporting excellent growth of the virus tumor tissue. Evidently it has all the materials necessary for the growth of this tissue, since stock cultures have been maintained to date for over 6 months on pure coconut milk with 1% agar. These materials, however, are not in the proper relative proportions as is shown by the fact that, with the addition of a small amount of medium no. 24, growth is increased. It is probable that some materials such as phosphate and nitrate are inadequate (3, 15) and become limiting after a short period of growth.

Discussion

VAN OVERBEEK has shown that indoleacetic acid is present in coconut milk and that more is extractable after autoclaving than before autoclaving (12). It has been shown that indoleacetic acid will stimulate the growth of the virus tumor tissue (10). However, the maximum stimulation caused by indoleacetic acid is about 20%, whereas the maximum stimulation caused by the addition of coconut milk is over 100% (table 1). The presence of indoleacetic acid in coconut

milk, therefore, does not explain the stimulation of virus tumor growth by this substance. This is the same conclusion reached by CAPLIN and STEWARD (4) in their work with carrot tissue. They found that the stimulation caused by the addition of coconut milk to the basal medium was of a different order of magnitude from that caused by the addition of indoleacetic acid. Starting with 4-mg. explants from carrot phloem tissue, they found that in 21 days indoleacetic acid caused a mean increase to 8.2 mg. but that coconut milk caused a mean increase to 184.0 mg.

On two points, however, the results with virus tumor tissue differ from those with carrot tissue. Explants of carrot were unable to survive when grown solely on coconut milk, whereas the growth of the virus tumor is excellent on this substrate. Carrot tissues reached their optimum growth when 15–20% coconut milk was present, in addition to the otherwise complete nutrient medium. A marked limitation in growth resulted from an increase in the concentration of the coconut milk over the optimum. In the case of the virus tumor tissue, the peak of the stimulation caused by the addition of coconut milk to the basal medium has not been reached in these experiments. The best growth was at 30% of coconut milk by volume, the highest concentration so far tested.

As a result of his work with *Datura* embryos, VAN OVERBEEK concluded that coconut milk contains three factors which influence growth, one of which is thermolabile, two thermostable. The results presented here with the virus tumor tissue suggest the possibility of at least two factors being involved, a thermolabile factor which is inhibitory to growth and a thermostable factor which is stimulatory.

Summary

1. The addition of coconut milk to a basal medium capable of supporting good growth of virus tumor tissue from *Rumex acetosa* results in a pronounced stimulation of growth, which is not due to indoleacetic acid.

2. Pure coconut milk is capable of supporting excellent growth of the virus tumor tissue. The carrot explants of CAPLIN and STEWARD will not grow on coconut milk alone. The other tissues discussed have not been tested on this substrate alone.

3. Autoclaved milk is much more stimulatory than sterile-filtered milk for the growth of virus tumor tissue. This suggests that there is a heat-labile factor (or factors) in the milk inhibitory to the growth of this tissue which partially negates the effect of the stimulatory substance. The stimulatory substance (or substances) is apparently heat-stable.

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CAROTENOIDS OF THE MUSHROOM CANTHARELLUS CINNABARINUS

FRANCIS HAXO

Introduction

Coloration attributable to carotenoid pigments has been reported in a wide variety of fungi (for a recent compilation refer to [3]), yet in only a few instances have the pigments been extracted and subjected to chromatographic analysis. Among the basidiomycetes the most thorough investigation to date is that carried out by WILLSTAEDT (**6**) on three species of *Cantharellus*. The pigment mixture from *C. cibarius* was found to consist mainly of beta-carotene; also present were lycopene, alpha-carotene, and two other carotenes, probably the delta and gamma isomers. *C. lutescens* and *C. infundibiliformis* contained, as the major component, an unknown carotenoid—its absorption spectrum is similar to flavorhodin and certain other yellow pigments—along with appreciable amounts of lycopene and possibly gamma-carotene.

In the present study the coloring matter of a fourth species, *C. cinnabarinus* ("cinnabar chantarelle"), has been investigated. The fruiting bodies of this small edible fungus vary in color from pink to red-orange, with the inner portions of the flesh usually being somewhat lighter in color than the outer. As described below the pigment extract was found to contain a mixture of at least five carotenoid pigments, only one of which, beta-carotene, appears to be present in the species investigated by WILLSTAEDT. The major component is apparently a new xanthophyll. The minor pigments, other than beta-carotene, have not been identified, but it is probable that some or all of these represent new or little-known carotenoids. The extracts also contain small amounts of phytofluene (**8, 9**), thus extending to the basidiomycetes the occurrence of this colorless C_{40}-polyene. Among the fungi phytofluene has previously been observed in *Rhodotorula rubra* (**1**) and *Neurospora crassa* (**7**).

Experimentation

CHROMATOGRAPHIC SEPARATION

The starting material consisted of 75 gm. of fresh, whole mushrooms collected in the Baltimore area. They were dehydrated with acetone, pulverized in a

mortar, and extracted repeatedly with acetone until the residue was almost colorless. The pigment from the combined extracts was transferred to petroleum ether (b.p. 30°–60° C.), concentrated to a solid residue, and dissolved in benzene. After saponification with methanolic KOH (10%), the benzene solution was washed free of alkali, dried with anhydrous sodium sulfate, and diluted with an equal amount of petroleum ether. The deeply colored pigment solution was adsorbed on a MgO column (Merck,

TABLE 1

Width (mm.)	Appearance	Designation
2.....	Yellow-orange	I
6.....	Colorless	
1.....	Pink	II
8.....	Colorless	
3.....	Purple	III
5.....	Colorless	
37.....	Deep red	IV
10.....	Pale orange (diffuse)	V
7.....	Colorless	
15.....	Pink	VI
40.....	Colorless	
8.....	Orange-yellow	VII
10.....	Fluorescent under ultraviolet	VIII

heavy powder, mixed with equal parts by volume of Celite) and developed with a mixture of petroleum ether and benzene (1:1) containing 1% acetone. The developed column showed the zones given in table 1 (the figures to the left denote the width of the respective zones on a column 45 × 225 mm.). The lower two zones overlapped, and these were collected in the filtrate, whereas the colored zones remaining on the column were eluted individually with a mixture of ethanol in petroleum ether-benzene. Those fractions to be studied further were purified by readsorption on small columns.

The major pigment—canthaxanthin

Most of the pigment was contained in Zone IV: subsequent chromatograms effected its separation from small amounts of a more weakly adsorbed orange-yellow zone, presumably an isomer of the major pigment. The isomer may be represented in the initial chromatogram by Zone V; however, the latter yielded only a very small amount of pigment, and this was discarded without further study. The major pigment was crystallized from a minimal volume of benzene by the slow addition, to excess, of methanol and by storage overnight in the cold. The yield of dried crystals was 0.9 mg., a lesser amount was recovered by recrystallization from the mother liquor. Microscopically the color of the individual crystals was a rather pale, reddish-brown. They were small, variable in size, and, for the most part, in the form of thin, elongated, rectangular plates (m.p. 218° [cor., sealed tube, filled with CO_2]). The preparation contained small amounts of colorless impurities.

At room temperature the dried crystals were essentially insoluble in methanol and ethanol, sparingly soluble in petroleum ether (b.p. 30°–60° C.) and hexane, more soluble in acetone, and still more soluble in benzene and carbon disulfide. When petroleum ether solutions were partitioned over aqueous methanol, the following distribution was noted: with 85% methanol, a trace of the pigment in the hypophase; with 90% methanol, less than half of pigment in hypophase; with 95% methanol, somewhat more than half of pigment in hypophase. The partition behavior was the same when either alkaline or acidic methanol was used. Petroleum ether solutions of the pigment gave a blue coloration with H_2SO_4 but not with H_3PO_4 or HCl (overnight treatment). These observations and adsorption behavior suggest that the pigment is one of the nonacidic, oxygen-containing (probably non-hydroxy) carotenoids. The name "canthaxanthin"

is proposed for this new xanthophyll in accordance with recent suggestions on nomenclature (2).

In all solvents tested the pigment showed a single broad absorption maximum in the visible spectrum centered at

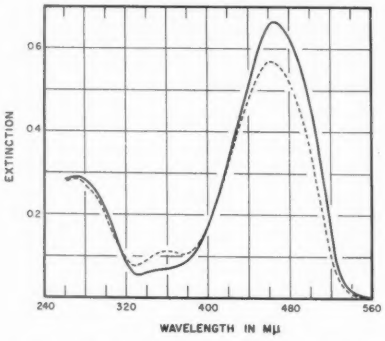

FIG. 1.—Extinction curves of canthaxanthin in hexane: ————, fresh solution of all-*trans* compound; – – – – – –, mixture of stereoisomers after iodine catalysis in light.

500 mμ in carbon disulfide, at 482 mμ in benzene, at 462 mμ in petroleum ether (30°–60° C.), and at 466 mμ in hexane. (A Beckman spectrophotometer was employed in all spectral determinations.) After iodine catalysis of the hexane solution in the light, the peak shifted to 461–462 mμ, and a "cis-peak" appeared at 360 mμ. Extinction curves in hexane of the all-*trans* pigment and the isomerization mixture are presented in figure 1. For this determination a portion of the crystals was shaken in acetone, and the dissolved pigment quickly transferred to hexane by dilution with water. The hexane solution was washed free of acetone and filtered over anhydrous Na_2SO_4.

MINOR PIGMENTS

Studies of the minor pigments were necessarily limited by the small quantities available, and none of these was obtained in crystalline form. The strongly adsorbed pigment of Zone I was discarded, it may merely represent altered pigment. The pigment from Zone II, when readsorbed on $Ca(OH)_2$, gave a complex chromatogram including six narrow, colored zones, which were discarded. From a diffuse pink zone situated below these closely packed zones was obtained a small amount of an all-*trans* pigment whose absorption spectrum was indistinguishable from that of canthaxanthin. When partitioned over 95% methanol, more than half of the pigment was in the hypophase; the pigment was predominantly epiphasic to 90% methanol. This pigment was not studied further.

The carotenoids obtained from Zone III and Zone VI were completely epiphasic to 85 and 90% methanol, and almost so to 95% methanol. Both pigments yielded a blue color when treated with H_2SO_4 but not with H_3PO_4. An extinction curve of the deep red pigment from Zone III is given in figure 2. The maxima and minima were at *520*, 512, *490*, 462 (inflection), 405, *384* mμ. With the addition of iodine the principal maximum at 490 mμ shifted to 487 mμ, and the first

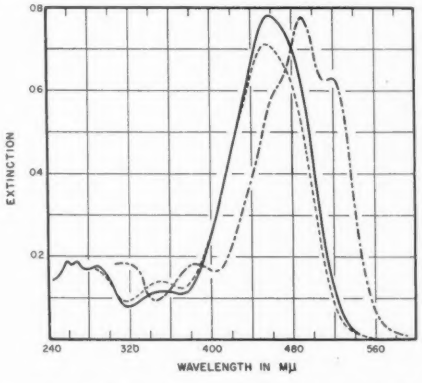

FIG. 2.—Extinction curves of two unidentified minor pigments in hexane: — — — — —, pigment III; ————, pigment VI; – – – – – –, pigment VI after addition of iodine.

maximum was replaced by an inflection at 517 mμ. In its spectral properties this pigment is somewhat similar to a red pigment isolated from purple bacteria (**5**); however, its identity has not been established.

Absorption curves of the pigment from Zone VI are characterized by their absence of fine structure, with a single broad absorption peak in the visible spectrum located at 494 mμ in carbon disulfide, 455 mμ in petroleum ether (b.p. 30°–60° C.), and 457 mμ in hexane (fig. 2). Isomerized hexane solutions show an absorption maximum at *454* mμ and a *cis*-peak at 350 mμ. The pigment resembles LEDERER's glycymerin (**4**) in its absorption spectrum but apparently differs in solubility.

The minor pigment contained in Zone VII displayed the beta-carotene spectrum, with maxima and minima in hexane as follows: *476*, 468, *450*, 426 (inflection) mμ (with iodine, *473*, 466, *448*, 425 [inflection] mμ). Its identity was confirmed by a mixed chromatogram with authentic beta-carotene.

The colorless fluorescent compound contained in Zone VIII is apparently phytofluene (**8, 9**), although with the small amount of substance available it could not be freed of colorless impurities and identified with certainty. The position of the maxima and minima (*366.5*, 357, *347*, 337, *329* mμ in petroleum ether) and the shape of the absorption spectrum are in agreement with impure preparations of phytofluene isolated from *Neurospora*.

The adsorption position of the *C. cinnabarinus* pigments relative to some known carotenes is given below, the pigments being designated according to their location in the zones of the initial chromatogram. For the mixed chromatograms the MgO-Celite mixture was employed as adsorbent and various concentrations of acetone in petroleum ether-benzene (1:1) as developer. Two per cent acetone was used to separate gamma-carotene and pigment VI, 5% acetone for pigment IV and lycopene, and 10%–30% to separate lycopene and pigment III.

pigment II
pigment III
lycopene
pigment IV (canthaxanthin)
gamma-carotene
pigment VI
beta-carotene

Summary

The edible mushroom *Cantharellus cinnabarinus* contains a complicated mixture of carotenoid pigments. The major component was isolated in crystalline form and named canthaxanthin. The only minor pigment identified with certainty was beta-carotene; three others have been characterized as to absorption spectra and partition behavior. A fluorescent substance, probably phytofluene, is present in the pigment extracts.

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CHROMOSOMAL ABERRATIONS IN ONION ROOTS FROM PLANTS GROWN IN AN ATMOSPHERE CONTAINING $C^{14}O_2$[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 619

J. M. BEAL AND N. J. SCULLY

Introduction

Several reports dealing with the effect of radioactive isotopes have appeared during the last 2 or 3 years. From these acounts it appears that perhaps any radioactive isotope that enters the plant may produce certain cytological aberrations. Chromosome fragments, chromosome bridges, and fragments at anaphase and chains of three or more chromosomes were observed in microsporocytes of *Tradescantia* when the plants were supplied with phosphorus 32 (**1**, **2**, **4**). Carbon 14 supplied to *Tradescantia* as $(NH_4)_2C^{14}O_3$ at different dilutions or differing activities induced chromatid and isochromatid breaks, chromatid exchanges, and chromatid deletions and exchanges in microspore nuclei (**5**). These induced changes appear to be similar in all essential respects and also to those induced by X-rays and slow neutrons (**6**).

During the last 2 years, in a co-operative project with Dr. E. M. K. GEILING and his associates, the senior author (**3**) has been investigating some of the histological effects of carbon 14 on the leaves of certain drug plants. More recently the junior author has grown carbon 14 plants from sets of the Ebenezer variety of onion in the special chamber at the Argonne National Laboratory; the root tips used in the present study were obtained from these plants.

Experimentation and observations

The Argonne nutriculture plant growth chamber has been designed especially for growing higher plants in a controlled environment in which carbon 14 can be supplied in gaseous form in the atmosphere and as ions in the nutrient solution. A temperature of $72° \pm 2°$ F. and a relative humidity of $55\% \pm 2\%$ were maintained throughout the course of the experiment.

The onion sets, $\frac{1}{4}$–$\frac{3}{8}$ inch in diameter, were planted in sand in the chamber on October 11, 1949, and grown with the nutriculture method but with the normal greenhouse atmosphere until October 20, 1949. On this date the chamber was hermetically sealed, and $C^{14}O_2$ was introduced into the atmosphere of the chamber. Additional quantities of $C^{14}O_2$ and $C^{12}O_2$ were introduced when required. The concentration of total carbon dioxide was allowed to vary from 0.03% to 0.1%. Within the hermetically sealed system the complete nutrient solution was pumped three times each day up into the gravel substrate. Unquestionably, some

[1] This work was supported in part by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago and the Atomic Energy Commission.

of the radiocarbon fixed by the plants was absorbed from the nutrient solution by the roots. The photoperiod was extended to 16 hours, by means of incandescent lights, from November 15, 1949, until termination of the growth period of the experiment on January 31, 1950. Controls of the same variety were grown in the greenhouse near the chamber under conditions of temperature, light, humidity, and nutrition as similar as possible to those prevailing in the chamber.

Root tips were collected at three different times, fixed in Navashin's solution, imbedded according to the tertiary butyl-alcohol method, sectioned at 10 μ, and stained chiefly in Haidenhain's hematoxylin.

The first roots were collected on November 30, 1949, when the chamber was opened, two plants removed, their root tips collected and fixed, and the plants then dried at 80° C. At this time it was noted that the old leaf bases that had comprised the original sets were shriveled and that the oldest of the green leaves which had developed during the period of growth in the chamber were just starting to swell at their bases, indicative of the onset of bulbing. The number and the size of roots developed on these plants were approximately the same as on corresponding control plants.

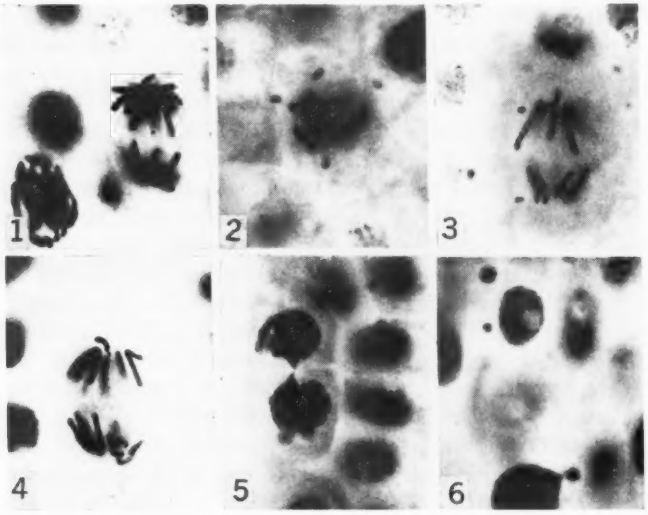
The dried plant material was ground to 40-mesh size and assayed for radioactivity with a thin-mica Geiger counter. The absolute activity (activity per gram dry weight as distinguished from "specific activity" which measures activity per gram of carbon) of this material was 44 μc/gm.

A second collection was made on December 13, 1949, when two more plants that showed the most advanced stage of bulbing were removed from the chamber. Most of the leaves on these two plants had died back, but evidences of bulbing were apparent nevertheless. The plants were air-dried for a period of 4 days, their dried leaves cut away, and the bulbs then stored at 40° F. until February 1, 1950. On removal from storage the bases of the bulbs were placed in water to permit root development. Although the bulbs were firm and normal in appearance, no roots emerged until March 10, when a single one appeared. This root, however, ceased elongation when it had reached a length of 1 mm. Additional roots began emerging on March 30 so that a total of five tips were obtained between April 5 and 10. On April 12 both bulbs were placed in sand and supplied with a complete nutrient solution. One of the plants soon died, but the other one grew slowly and on May 15 had produced a total of six leaves. One of them showed a band of chlorophyll-deficient tissue, 1–2 mm. in width, extending the full length of the leaf. No chlorophyll-deficient tissue was noted to occur in control material.

The four remaining bulbs appeared mature and were removed from the chamber on January 31, 1950, when the growth phase of this part of the experiment was terminated. They were air-dried for 4 days and stored at 40° F. until April 5. Their bases were then immersed in water. Four days later two of the bulbs had decayed without forming a single root, but the two others produced a total of six roots by April 9. After these roots were collected and fixed, the surviving bulbs were transferred to sand and maintained with a complete nutrient solution. Although both produced leaves and additional roots, they showed such marked symptoms of dying by May 10 that they were oven-dried, ground to 40-mesh size, and then assayed for radioactivity. The absolute activity recorded was 46 μc/gm.

Root tips were collected from the control plants on November 30, 1949, and handled in the same manner as those obtained from the experimental plants.

All the root tips obtained from the plants supplied with C^{14} have been sectioned, stained, and critically studied. In only three of the roots were mitotic figures observed. The remaining roots showed aberrations of one kind or another which were most easily recognized at anaphases and telophases, since fragments, bridges, and micronuclei may be easily seen at these stages. Fragments, most commonly in pairs, were of frequent occurrence (figs. 2, 3), indicating a chromosome break to produce equal-sized fragments. In several instances,



FIGS. 1–6. Mitosis in onion root tips.—Fig. 1, mid-prophase and late anaphase stages from control with no signs of aberrations. Figs. 2–6, from roots of bulbs grown in atmosphere supplied with carbon 14. Fig. 2, anaphase stage showing five fragments. Fig. 3, later anaphase stage showing pair of similar fragments. Fig. 4, anaphase stage showing three fragments. Fig. 5, late telophase with bridge nearly severed by developing cell plate. Fig. 6, upper cell with two micronuclei near large nucleus; lower cell with small fragment being pinched off.

had made a limited amount of growth, and this apparently as a consequence of elongation of the cells in the embryonic roots present in the young bulbs instead of as a result of cell divisions. Although two of these roots showed evidences of lateral-root formation, neither dividing cells nor micronuclei were observed in them.

Dividing nuclei were numerous in the three roots previously mentioned. In fact, they were approximately as frequent as in the controls. Many nuclei however, single fragments have been seen (fig. 4), thus indicating a chromatid break. Bridges have been seen occasionally in late anaphases and telophases (fig. 5). Here the bridging chromosome has been pinched through by the developing cell plate. Micronuclei were also of relatively frequent occurrence (fig. 6). In contrast mitosis appeared to be normal in all the figures observed in the roots of controls, with neither fragments nor bridges occurring (fig. 1).

These effects of assimilated C^{14} on

chromosomes in intact growing onion plants confirm results previously reported for this element under different experimental conditions as well as for other radioactive substances. Since the carbon 14 becomes a part of the basic organic compounds, both in living and in nonliving compounds, its effects should continue indefinitely, and the cytogenetic consequences of the radioactivity should be cumulative; and although too heavy doses of carbon 14 might be lethal to the plant or markedly affect its growth and development, relatively light ones probably would be much less harmful and result in cytogenetic effects of interest and significance in producing biological variations. Additional studies are under way, and further reports are planned.

Summary

1. Sets of the Ebenezer variety of onion grown in the special nutriculture chamber at the Argonne National Laboratory under controlled temperature and humidity conditions had their natural atmospheric carbon dioxide supply supplemented by the addition of $C^{14}O_2$ so that the total concentration varied from 0.03% to 0.1%. The photoperiod was extended to 16 hours by means of incandescent lamps.

2. One leaf from a plant of approximately a dozen grown showed a chlorophyll-deficient streak along its entire length. There were no chlorotic streaks in control plants.

3. Roots from the carbon 14 plants showed both chromosome and chromatid breaks and fragments in dividing cells, bridging, and micronuclei. No aberrancies were observed in dividing cells of control roots.

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CURRENT LITERATURE

Embryology of Angiosperms. By P. MAHESHWARI. New York, Toronto, London: McGraw-Hill Book Co., 1950. Pp. x+451. $6.00.

MAHESHWARI's book is the only one in English on this subject to appear since COULTER and CHAMBERLAIN's *Morphology of Angiosperms*, which dates from the first decade of this century. The amount of work done since that time, and even since the publication in 1929 of SCHNARF's *Embryologie der Angiospermen*, more than justifies the appearance of a new book. MAHESHWARI's competence in the field of angiosperm embryology has long been known in this country, particularly as a result of the appearance of many review articles in American journals. He is, then, an appropriate person to write such a text.

The book deals with embryology in the broad sense of the term, covering microsporogenesis, development of the pollen grain and tube, megasporogenesis, megagametogenesis, fertilization, endosperm development, and embryogeny. In addition, apomixis and polyembryony are considered, and the history of the subject, the relation of embryology to taxonomy, experimental embryology, and theoretical implications are briefly discussed.

The presentation of the factual material is clear and well illustrated. MAHESHWARI makes no attempt to treat the material exhaustively, but his choice of representative species and of cited papers gives a satisfactory picture of the range of variation in developmental pattern that occurs and of the many workers throughout the world. The book will serve admirably to acquaint a student with the patterns of angiosperm reproductive development and to provide the research worker with a starting point in working out the range of variation that may occur in one area or in relating a particular sequence of development to the patterns that are known to occur.

To this reviewer the brief consideration of theoretical problems and the relative lack of evaluation of contradictory interpretations are among the weakest portions of the book. Also weak is the limitation of consideration of problems of experimental embryology to those few areas in which some work has been done without suggesting additional highly interesting but baffling problems which are waiting to be attacked by some ingenious individual: physiological problems such as the nutrition of the gametophytes and endosperm, the actual role of the endosperm and reasons for its failure in certain cases, the directive mechanism for pollen-tube growth, and the morphogenetic problems involved in the development of the zygote and embryo, including the potentialities of various cells and the degree of predetermination of the ultimate fate of cells—comparable to work done in animal embryogeny. The problems in these areas are admittedly the knottiest confronting the worker in angiosperm embryology, and in many cases no satisfactory answer can be reached; but the problems exist and should not be passed over too lightly. Technical terms are used throughout, in many cases without explanation, and, as there is no appended glossary, the student may be somewhat at a loss in certain instances to understand the material adequately. This seems a definite lack in a book evidently intended as a standard text.

The book, then, though perhaps somewhat deficient on the interpretative side, is a very workmanlike and satisfactory compilation of representative information concerning the embryology of the angiosperms.—B. F. PALSER.

Praktikum der Zell- und Gewebephysiologie der Pflanze. By SIEGFRIED STRUGGER. 2d ed. Berlin, Göttingen, Heidelberg: Springer Verlag, 1949. Pp. viii+225+148 figs. Unbound, DM.24.00; cloth, DM.27.60 (approx. $6.50 and $7.45).

The Springer series of "Pflanzenphysiologische Praktika" promises to be of great value in efforts to introduce modern experimental procedures in laboratory courses in plant physiology and experimental cytology. The present book, Volume II of the series, starts out with general directions for the preparation of plant material for experiments with cells and tissues and for the use of optical instruments (light- and dark-field observation, fluorescence and phase contrast analyses). The remainder of the volume contains descriptions of an abundance of experiments either for practicing special techniques (e.g., measurement of viscosity; vital staining) or for studying individual constituents of the cell (chloroplasts, chondriosomes, vacuoles) or various processes (uptake and transport of solutes; movements). The various types of plasmolysis and their significance for the understanding of the properties of the cytoplasm and the cell sap receive a thorough and detailed treatment. The text is profusely illustrated with drawings and photographs, the latter reproduced on calendered paper.

The units of which the book is built up are individual experiments. Although theoretical interpretations accompany the descriptions of experiments, the author does not attempt a monographic integration. About 685 references to pertinent literature, however, make the work very valuable as a lead to complete information even in theoretical aspects.—POUL LARSEN.